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Distribution and Management of and Soybean Resistance to Calonectria Crotalariae, the Causal Pathogen of Red Crown Rot of Soybean.

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Distribution and management of and soybean resistance to *Calonectria crotalariae*, the causal pathogen of red crown rot of soybean

Berner, Dana Kent, Ph.D.

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DISTRIBUTION AND MANAGEMENT OF AND SOYBEAN RESISTANCE TO *CALONECTRIA CROTALARIAE*, THE CAUSAL PATHOGEN OF RED CROWN ROT OF SOYBEAN

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and Agricultural and Mechanical College
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

in

The Department of Plant Pathology and Crop Physiology

by

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER V

LIST OF FIGURES

Chapter II

Chapter III

Chapter IV

ABSTRACT

Distribution and management of and soybean resistance to *Calonectria crotalariae*, the causal pathogen of red crown rot of soybean

The national, local, and in-field distribution of *Calonectria crotalariae* (Loos) Bell and Sobers, as reflected in the distribution of Cylindrocladium black rot (CBR) of peanut, red crown rot (RCR) of soybean and microsclerotia densities in soil, respectively, are described. The current distribution of RCR in Louisiana is probably due to the introduction of the pathogen on indigo (*Indigofera tinctoria* (L.)) and to subsequent movement by man. RCR has been reported in 17 Louisiana parishes.

In field studies, delayed soybean planting was efficacious in reducing RCR incidence. Effects of delayed planting on yield were maturity group dependent and confounded with RCR effects. A statistical model was constructed that predicted 50% yield loss at 100% RCR symptomology on a susceptible cultivar.

Associations between weed infestation and RCR incidence were observed in the field, and indicated that the amino acid biosynthesis-inhibiting herbicides glyphosate, imazaquin, and chlorimuron ethyl may have fungitoxic properties. Glyphosate significantly reduced *in vitro* *C. crotalariae* colony area, at field-use rates. Preplant applications of glyphosate also reduced RCR incidence in two field studies. To study the effects of these materials on microsclerotia production, a technique was developed which employed fungal culture on solid medium in combination with colorimetry and a standard curve to indirectly count microsclerotia. Imazaquin significantly reduced production microsclero-

tia in vitro. Chlorimuron ethyl was less effective than imazaquin. Simulated repeat applications of these materials were effective in restricting colony growth. In field studies, RCR incidence was not significantly reduced by preplant imazaquin applications. Long-term cumulative effects of the herbicide on RCR are postulated.

A procedure was developed and used over a six year period to evaluate cultivars for reaction to RCR. These trials showed clear differences in RCR reaction among cultivars. Resistant cultivars can be selected within any maturity group. Location instability of RCR reaction on several cultivars indicates the pathogen population may be heterogenous. Two mechanisms of resistance are proposed.

CHAPTER I

DEPARTMENT OF PLANT PATHOLOGY AND CROP PHYSIOLOGY

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Sigrid Vargonis 4/17/91

Distribution and Management of Red Crown Rot of Soybean in Louisiana

ABSTRACT

Pathogen distribution, soybean cultivar resistance, and influence of planting date on red crown rot (RCR) incidence and yield loss were investigated. National and statewide distribution of the causal fungus, Calonectria crotalariae, suggests the organism may have been introduced into the United States by the dye crop indigo (Indigofera tinctoria L.). Movement of fungal propagules from the former indigo plantations by flood water may account for the current distribution of the disease in 17 soybean-producing parishes in Louisiana. Using the presence of perithecia as a means of assessing disease incidence, the maturity group VII cultivars were relatively tolerant to RCR. While delayed planting resulted in decreased RCR incidence, this relationship was significant only for the susceptible maturity group V cultivar, 'Bedford'. Both RCR incidence and planting date had a significant effect on 'Bedford' yield and when these effects were statistically analyzed, a 50% yield reduction was predicted at 100% RCR incidence. Yield of the more tolerant maturity group VII cultivar, 'Braxton', was not significantly affected by RCR but was reduced by delayed planting.

INTRODUCTION

Red crown rot (RCR) of soybean (Glycine max (L.) Merr.) is caused by the fungus Calonectria crotalariae (Loos) Bell and Sobers (imperfect stage Cylindrocladium crotalariae) (2). The organism was first described on peanut (Arachis hypogea L.) in 1966 in Georgia causing the disease known as black root rot (2). In 1972, the fungus was identified as pathogenic on soybean (12), and has been variously described as red crown rot, Cylindrocladium black rot and black root rot (2,3,11,12). Calonectria crotalariae also has been reported as pathogenic on papaya (Carica papaya L.) (10), koa (Acacia koa A. Gray) (1) and blueberries (Vaccinium corymbosum L.) (9), and has been found to be pathogenic on indigo (Indigofera tinctoria L.) in Louisiana (G. T. Berggren, personal communication) In peanut-growing areas the organism is thought to be transported through infested seed (6), plant debris, and farm machinery (13), but little is known about its movement and distribution in areas where peanuts are not grown. The initial report of RCR in Louisiana occurred in 1976 in St. John the Baptist Parish (H. K. Whitam, personal communication) which coincided with a shift in cultivation from cotton (Gossypium hirsutum L.) and sugarcane (Saccharum officinarum L.) to soybean. As soybean production acreage increased in other parishes, reports of RCR became more numerous and by 1981, the disease had been reported in five parishes (3).

The fungus overwinters in soil and crop debris as irregularly-shaped microsclerotia, 35-425 μm in diameter (11,13). Microsclerotia, the primary source of inoculum (13), germinate in the spring and infect susceptible host plant roots. Symptoms of RCR generally do not appear until the R5 (beginning pod fill) growth stage (5) or later (3) which

makes early disease assessment difficult. Initial symptoms appear as a very distinct interveinal chlorosis of the uppermost leaves. As the disease progresses, affected leaves dry and may fall off and frequently, whole plants will desiccate and become defoliated. Due to the aggregated distribution of the disease, patches of several symptomatic plants surrounded by apparently-healthy plants are commonly seen. As the leaves of diseased plants are shed, the amount of disease becomes increasingly difficult to assess based on canopy symptoms since surrounding green foliage obscures the defoliated plants. Because of the late onset of symptoms, the clustered distribution of symptomatic plants in the field, and the lack of information on distribution in soybean growing areas, it is difficult for a grower to plan a financially sound and timely control strategy. Chemical control measures are generally not cost effective, since they necessitate extensive soil fumigation, and the only reliable cultural practices have been rotation with non-host crops and/or the use of somewhat resistant cultivars (8). This research was conducted to: 1) examine the national, local, and in-field distribution of the pathogen to allow growers to predict whether they would be likely to encounter RCR and where in the field the disease might be most severe, 2) devise a method of disease assessment more accurate than canopy symptomology and to use this method to evaluate soybean cultivars for disease resistance, and 3) determine whether the initial flush of microsclerotial germination in the spring might be avoided by delayed planting and what influence that practice might have on yield.

MATERIALS AND METHODS

A review of the literature on the national distribution of C. crotalariae and the distribution of host plants was conducted. In the spring of 1985, a pamphlet describing RCR was distributed to Louisiana county agents who were asked to report any occurrence of the disease. Agents unsure of the diagnosis were asked to contact the Louisiana Cooperative Extension Service, Louisiana State University, Baton Rouge for confirmation.

To study the in-field distribution of the disease, a field with a history of RCR was divided into 80 rectangular plots, 12.2 m X 6.1 m. Relative land elevations, measured with a surveyor's level, were taken at twelve sites/plot (three measurements along each plot boundary) and a map of contours of 3.0 cm elevations was constructed. From the contours, in-field water drainage patterns were identified. Soil samples were taken from each field plot and microsclerotia were isolated from the soil by the method described by Phipps, et al. (11). Those field plots which contained an average density of greater than 10 microsclerotia/g of dry soil, which indicated the more clustered concentrations in this field, were marked on the contour map. Moisture content, determined from a subsample of soil from each plot, and average plot elevation were analyzed by multiple regression techniques for their effect on microsclerotial density.

In addition to leaf symptomology, RCR is characterized by the production of bright red perithecia at the base of the plant. This symptom appears at about the same time as the leaf symptoms and is a more stable symptom because of RCR induced defoliation. For both the

cultivar evaluation trials and planting date studies, RCR was assessed using random samples of a linear m of row in each plot. Within this sample, the total number of plants and the number of plants bearing perithecia were counted. Two-to-eight random samples were taken per plot, depending on plot size and each plot was sampled at three times after the R5 growth stage. Ratings for each plot were calculated by adding the number of perithecia-bearing plants from each sample and dividing by the total number of plants from each sample. A mean plot percentage of diseased plants was then arrived at by averaging these ratings over the three sampling times.

Cultivar evaluation trials were conducted during the 1985 and 1986 growing seasons in a field known to be heavily infested with C. crotalariae. Cultivar plots were two rows X 6.1 m in size and each cultivar was replicated four times. Two random samples were taken from each plot according to the method described above. Plots were harvested at respective maturities and yields were calculated based upon 13% moisture content.

In 1986, a study was conducted to determine whether planting date might be used to manage the disease. Since disease development apparently depends on germinating microsclerotia contacting roots of a susceptible host, the impact from an early flush of microsclerotial germination might be reduced by delaying planting. For this study, a relatively susceptible group V cultivar, 'Bedford', and a relatively resistant group VII cultivar, 'Braxton', were used. An infested field was planted at five different planting dates: May 23, June 2, June 13, June 20, and June 26. Each plot, as defined by cultivar, planting date, and replication, was 24.4 m long and contained four rows spaced 94 cm

apart. The cultivars were planted continuously across the four replications to facilitate harvest. At maturity, the middle two rows of each plot were harvested and yields were numerically adjusted to kg/ha at 13% moisture content. Each plot was rated for disease incidence by taking eight samples of one linear m of row per plot and calculating per plot percentages as previously described. Plots were sampled at three different times and mean plot percentages were calculated and statistically analyzed using linear and multiple regression techniques. To estimate percentage yield loss attributable to the disease, planting date was numerically held constant at day 143 and RCR incidence was numerically varied between 0 and 100%. A FORTRAN program using the expanded regression model predicted yields at each percent RCR incidence. The predicted yields were then converted to a percentage yield loss, using the calculated yield at 0% RCR as the zero loss standard.

RESULTS AND DISCUSSION

Geographical Distribution

To date, 17 Louisiana soybean-producing parishes have confirmed incidence of RCR and most of the parishes currently reporting C. crotalariae-infested fields lie along the Mississippi River (Fig. 1.1). In the early-to-mid-1700's, the East Indies Company introduced the dye crop indigo (Indigofera tinctoria L.) into Louisiana, North Carolina, South Carolina, Georgia, and Virginia (4,7). Indigo in Louisiana is an alternative host of C. crotalariae (Berner, unpublished data) and those states into which the crop was introduced also report problems with the organism, either on peanut (2,12) or soybean (3,8,14). In peanut-producing areas, the organism probably was spread further by infested seed

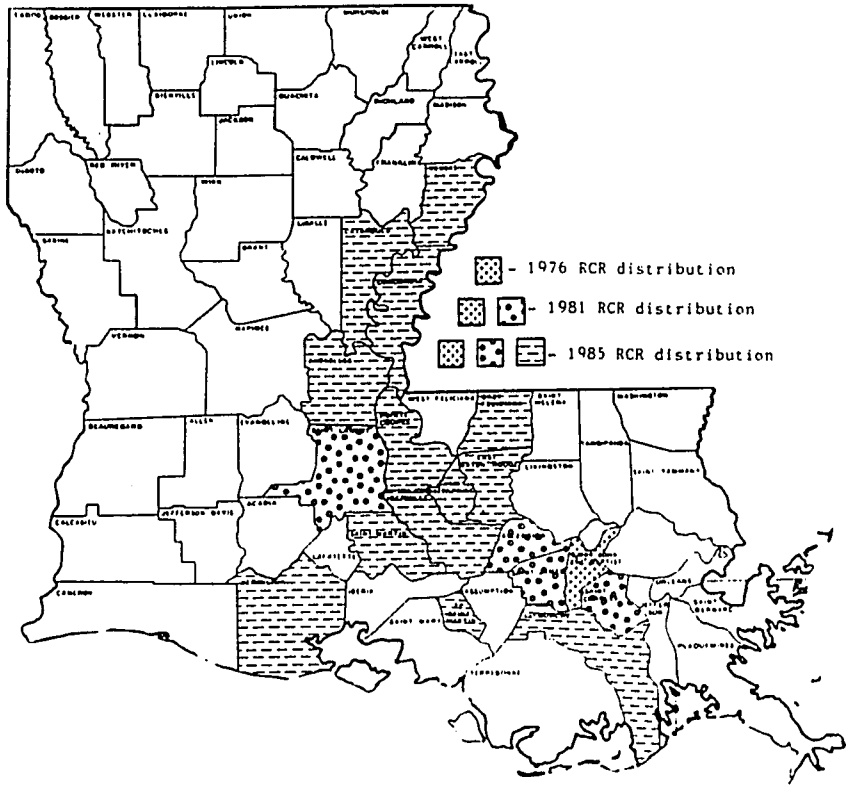

Fig. 1.1 Distribution of red crown rot in Louisiana.

(6) and plant debris (13). A possible explanation of the distribution of the disease in Louisiana is that the organism was moved by river flood waters out of the indigo plantations and into adjacent parishes. The indigo plantations were located primarily along the Mississippi River due to the need for water in the processing of dye, and because the River was the primary avenue of transportation. During this era the River was unleveed, and flooding was common and extensive. After indigo production ceased and levees were built along the River, the current pattern of pathogen distribution may have been established. The organism may have then survived as an unnoticed component of the soil microflora until acreage planted to soybean increased in the late 1970's.

In-field Distribution

Multiple regression of microsclerotial density (MS) on relative plot elevation (RPE) and soil moisture content (SMC) produced the following model: MS = 40.62 -10.79*RPE - 1.03*SMC ; R^2 = 0.07, PR>F = 0.05. Although the fit of the model was poor, the regression was significant as were the slope estimates, P=0.06 for RPE and P=0.02 for SMC. The slope for relative plot elevation was negative, indicating the positive influence of lower elevations on increased concentrations of microsclerotia. The negative slope for soil moisture content is inexplicable at present. The poor fit of the model is probably largely attributable to averaging individual site relative elevations over whole plots. The contour map of the study field, built from individual site elevations, is presented (Fig. 1.2). Arrows on the map indicate probable surface runoff patterns and the shaded areas indicate those plots which contained an average density greater than 10 microsclerotia/g of dry soil. Greater concentrations of initial inoculum were

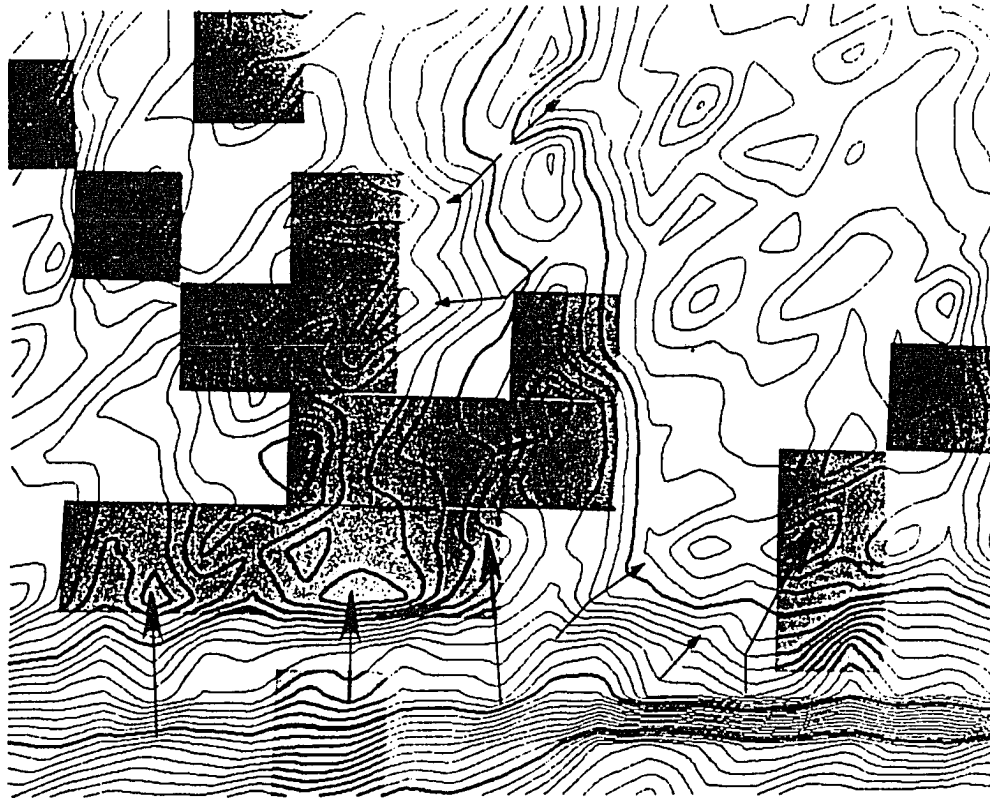

Fig. 1.2. Field elevation contours in 3.0 cm intervals with probable surface runoff patterns (arrows) and microsclerotia densities greater than 10/g dry soil (shaded) indicated. Data were collected at the Robert farm, Burnside, LA in the Fall of 1985.

found in low-lying areas of the field. Krigsvold et al. (8) reported a similar pattern in runoff from a C. crotalariae-infested peanut field affecting adjacent soybeans. If RCR was a suspected disease problem, low-lying areas would appear to be likely "hot spots" of infestation. In addition, surface runoff distribution of microsclerotia lends credence to the theory of dissemination of RCR in Louisiana by river flood water.

Cultivars

Maturity group VII cultivars tended to be more resistant than cultivars of other maturity groups (Table 1.1), while the highly susceptible cultivars, 'Hartz 6383', 'Centennial', and 'Forrest', are grouped at the other end of the scale. Analysis of variance of maturity group differences indicated that group VI soybeans were the most susceptible followed by maturity group V. Group VII soybeans were the most resistant. Since one of the growing seasons involved in this study was unfavorable for disease development, the differences between the cultivars/maturity groups is probably greater than indicated. The group V and VI soybeans, in general, appear to be relatively susceptible to the disease, while the maturity group VII soybeans are considerably more resistant. The apparent differences in resistance among maturity groups V, VI and VII are probably more related to phenological escape than to genetic resistance. However, differences within each maturity group indicate that the cultivars tested in these trials may possess some genetic resistance.

Planting Dates

The overall effect of planting date on RCR incidence demonstrates that delayed planting generally reduces RCR incidence (Fig. 1.3a). When

Table 1.1. Cultivar averages of red crown rot (RCR) rating (% incidence) and yield (kg/ha) from cultivar trials during 1985 and 1986 at the Robert farm, Burnside, LA.

CULTIVAR	MATURITY GROUP	RCR RATING (%)	YIELD (KG/HA)
HARTZ 6383	6	27.8	2768.6
CENTENNIAL	6	20.1	2950.5
FORREST	5	18.5	2425.1
RINGAROUND 680	6	16.9	3213.2
WILSTAR 550	5	16.4	2458.8
COKER 156	6	14.9	3065.0
DAVIS	6	14.0	2916.8
BRAGG	7	13.0	3065.0
DELTAPINE 506	6	12.8	3179.6
DELTAPINE 105	5	12.4	3058.3
ASGRO 5474	5	12.2	2660.9
HARTZ 7126	7	11.7	3105.5
DELTAPINE 345	5	11.7	2681.1
BEDFORD	5	11.4	2479.0
TRACY M	6	11.2	2863.0
COKER 237	7	9.3	3489.4
WRIGHT	7	8.9	3401.9
BRAXTON	7	8.3	3745.4
WILSTAR 790	7	7.5	3354.7
RANSOM	7	6.9	2748.4
$HSD_{.05}$		14.7	1044.1

the individual cultivar effects are separated (Figs. 1.3b and 1.3c), the susceptible cultivar 'Bedford' accounts for most of the fit and significance of the overall regression. The decline in incidence for 'Braxton' is not significant, and there was only a low level of disease throughout the planting season.

Yields of 'Braxton' and 'Bedford' show markedly different responses to planting date (Figs. 1.4a and 1.4b). Yields for 'Braxton' peak for planting on or about day 153 while 'Bedford' yields are relatively high at the early planting date (day 143) and at the last planting date (day 177). This "tailing up" effect at the later planting dates is likely due to a decrease in RCR pressure. If the regression lines (Figs. 1.3c and 1.3b) are overlaid, the point at which the two lines cross is the planting date at which yield begins to increase.

However, such yield-planting date regressions also contain the effect of RCR. In Tables 1.2 and 1.3, planting date and RCR effects on yield are statistically separated. Table 1.2 presents the analysis of variance for the resistant cultivar, 'Braxton', taking into account only planting date effects. The linear effect of date, expressed as day of year, is highly significant, while the quadratic effect is significant only at P=0.07. All slope estimates are significant at P=0.09. The overall fit of the regression (0.39) is significant at P=0.015. When the effect of RCR was added to this model, the overall fit increased slightly to 0.44, but the significance declined slightly to P=0.02. The effect of RCR was not significant (P=0.25). With this effect in the model, all slope estimates were not significant. RCR had no apparent effect on yield of the more resistant cultivar, and the opti-

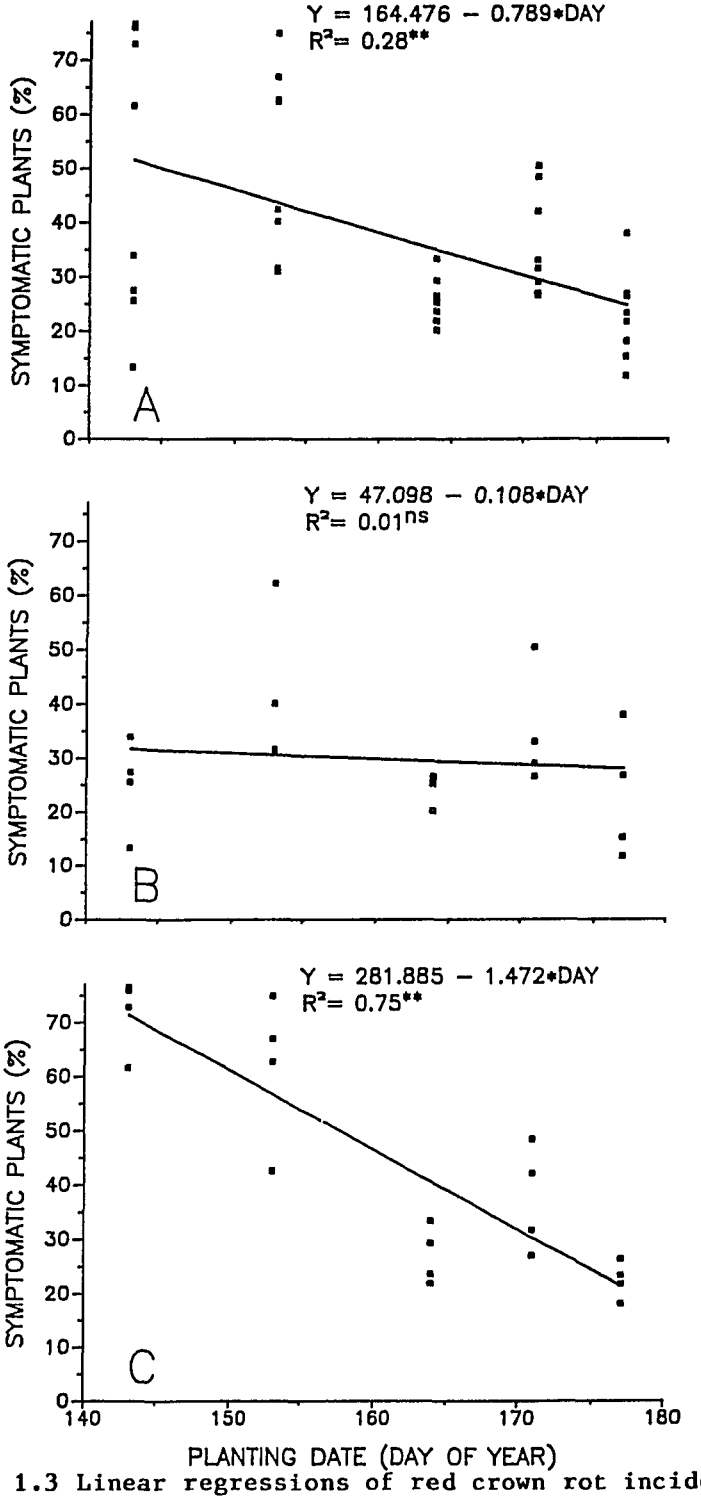

Fig. 1.3 Linear regressions of red crown rot incidence on planting date A) Overall effect of planting date on red crown rot incidence for the cultivars 'Braxton' and 'Bedford'. B) Effect of planting date on red crown rot incidence for the cultivar 'Braxton'. C) Effect of planting date on red crown rot incidence for the cultivar 'Bedford'.

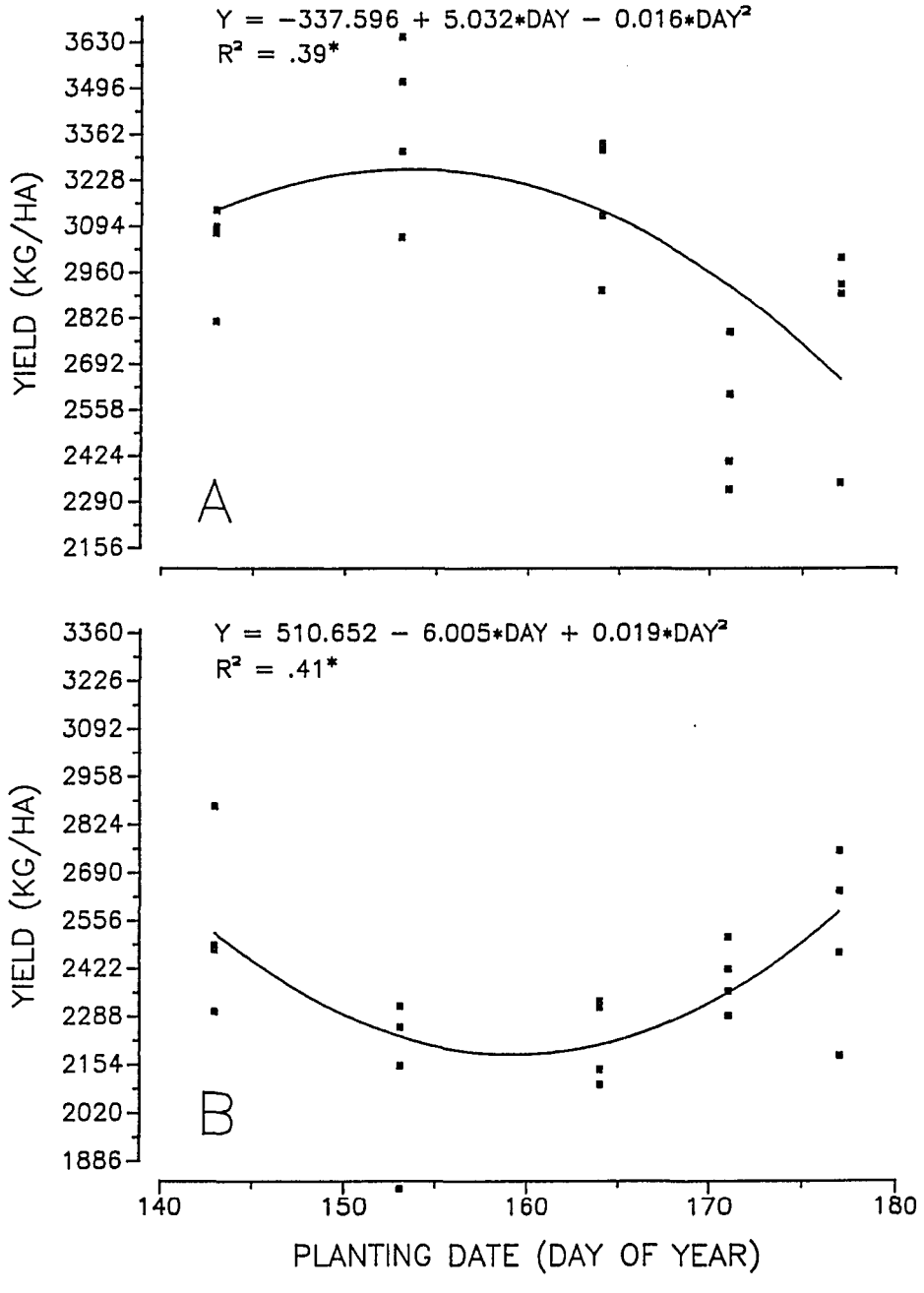

Fig. 1.4. Polynomial regressions of yield on planting date.
A) Effect of planting date on yields of the cultivar 'Braxton'.
B) Effect of planting date on yields of the cultivar 'Bedford'.

Table 1.2. Analyses of variance for the regressions of yield on planting date (DAY) and red crown rot (RCR) for the cultivar 'Braxton'.

MODELS

$YIELD = BO + B1*DAY + B2*DAY^2$

$R^2 = .39$ PR>F = 0.0154

SOURCE	DF	TYPE I SS	PR>F
DAY	1	144.018	0.0160
DAY*DAY	1	72.860	0.0741

PARAMETER	ESTIMATE	PR>T
INTERCEPT	-337.5951	0.1427
DAY	5.0316	0.0859
DAY*DAY	-0.0164	0.0741

$YIELD = BO + B1*DAY + B2*DAY^2 + B3*RCR$

$R^2 = .44$ PR>F = 0.0234

SOURCE	DF	TYPE I SS	PR>F
DAY	1	144.018	0.0155
DAY*DAY	1	72.860	0.0719
RCR	1	28.089	0.2489

PARAMETER	ESTIMATE	PR>T
INTERCEPT	-254.7509	0.2797
DAY	3.8502	0.2028
DAY*DAY	-0.0125	0.1897
RCR	1.3065	0.2489

Table 1.3. Analyses of variance for the regressions of yield on planting date (DAY) and red crown rot (RCR) for the cultivar 'Bedford'.

MODELS

YIELD = B0 + B1*DAY + B2*DAY^2

R^2 = .41 PR>F = 0.012

SOURCE	DF	TYPE I SS	PR>F
DAY	1	1.0823	0.7235
DAY*DAY	1	95.9207	0.0035

PARAMETER	ESTIMATE	PR>T
INTERCEPT	510.389	0.0026
DAY	-6.005	0.0036
DAY*DAY	0.019	0.0035

YIELD = B0 + B1*DAY + B2*DAY ^{2} + B3*RCR

R^2 = .53 PR>F = 0.009

SOURCE	DF	TYPE I SS	PR>F
DAY	1	1.0823	0.7013
DAY*DAY	1	95.9207	0.0020
RCR	1	28.6947	0.0615

PARAMETER	ESTIMATE	PR>T
INTERCEPT	516.389	0.0011
DAY	-5.647	0.0035
DAY*DAY	0.017	0.0054
RCR	-1.787	0.0615

mum planting date, as predicted from the reduced model in Fig. 1.4a, was day 153 (June 2).

The linear effect of planting day is not significant while the quadratic effect is highly significant (P=0.724 and 0.003, respectively) for 'Bedford' (Table 1.3). All of the slope estimates are highly significant. The overall fit is 0.41 with a PR>F = 0.012. When the effect of RCR is added to the model, both the overall fit and significance increase, to R^2=0.53 and P=0.009, respectively. The effect of RCR is significant at P=0.06, and all of the slope estimates remain significant. RCR as well as planting date have a significant effect on the yields of the susceptible cultivar.

To estimate the magnitude of the RCR effect, RCR was set equal to zero in the expanded model (Table 1.3), and the resulting predicted yields without the effect of RCR were plotted against predicted yields containing the RCR effects (Fig. 1.5). The difference between the two curves may be interpreted as the difference in yield attributable to the level of RCR in this study. To estimate the effects of different levels of RCR on yield, RCR in the expanded model was tested at varying percentages. From 0-to-20% RCR incidence predicted yield is reduced 539 kg/ha (Fig. 1.6). From 20-to-40%, however, yield is reduced by only 269 kg/ha. The effects of RCR are quite pronounced from 0-to-20% incidence above which there appears to be a "buffered" effect on yield loss. However, regardless of RCR incidence, the optimum planting date was early (day 143 or May 23 in this study).

From 0-to-20% and 75-to-100% disease incidence, pronounced effects on percentage yield loss in 'Bedford' were predicted (Fig. 1.7). From

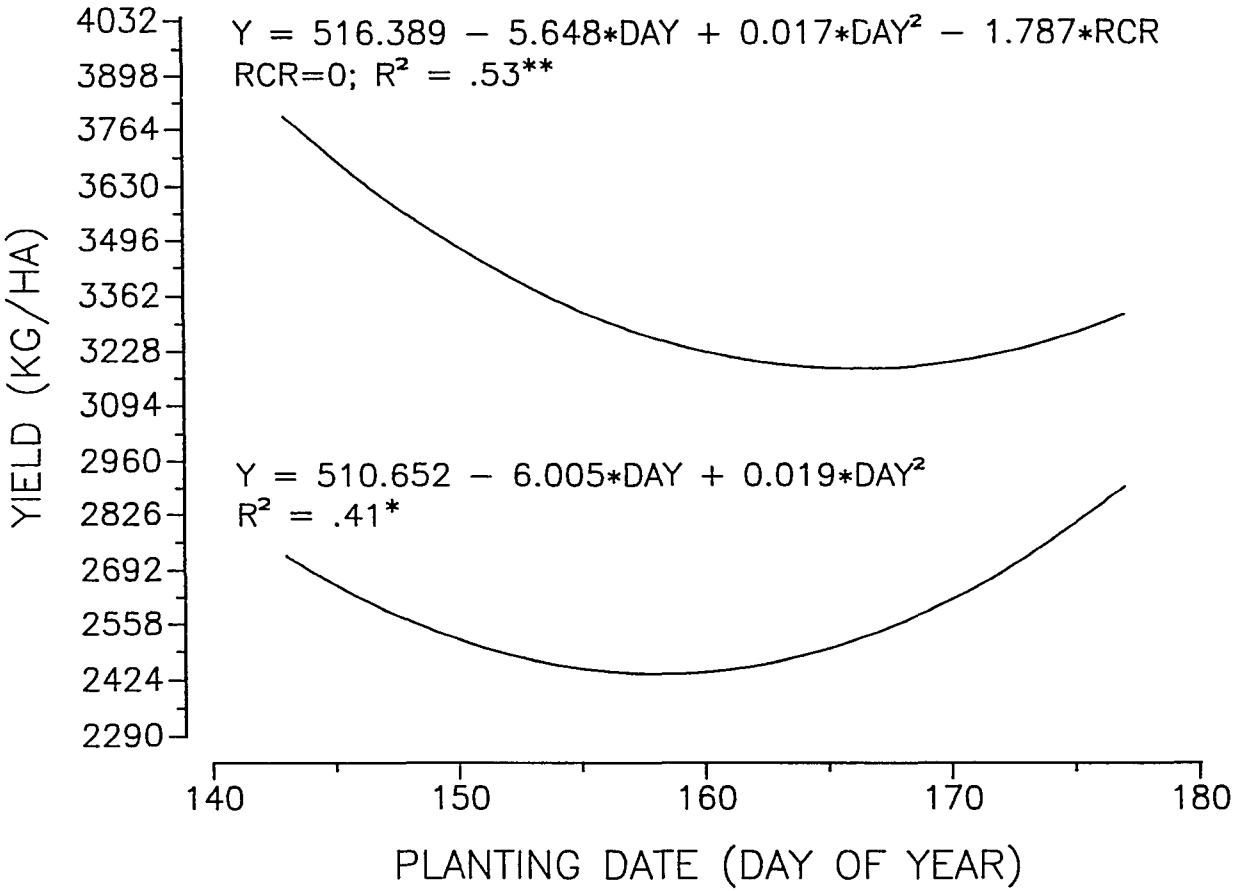

Fig 1.5. Polynomial and multiple polynomial regressions of yield on planting date for the cultivar 'Bedford'. Lower line-predicted yields with the effects of red crown rot. Upper line-predicted yields without the effects of red crown rot.

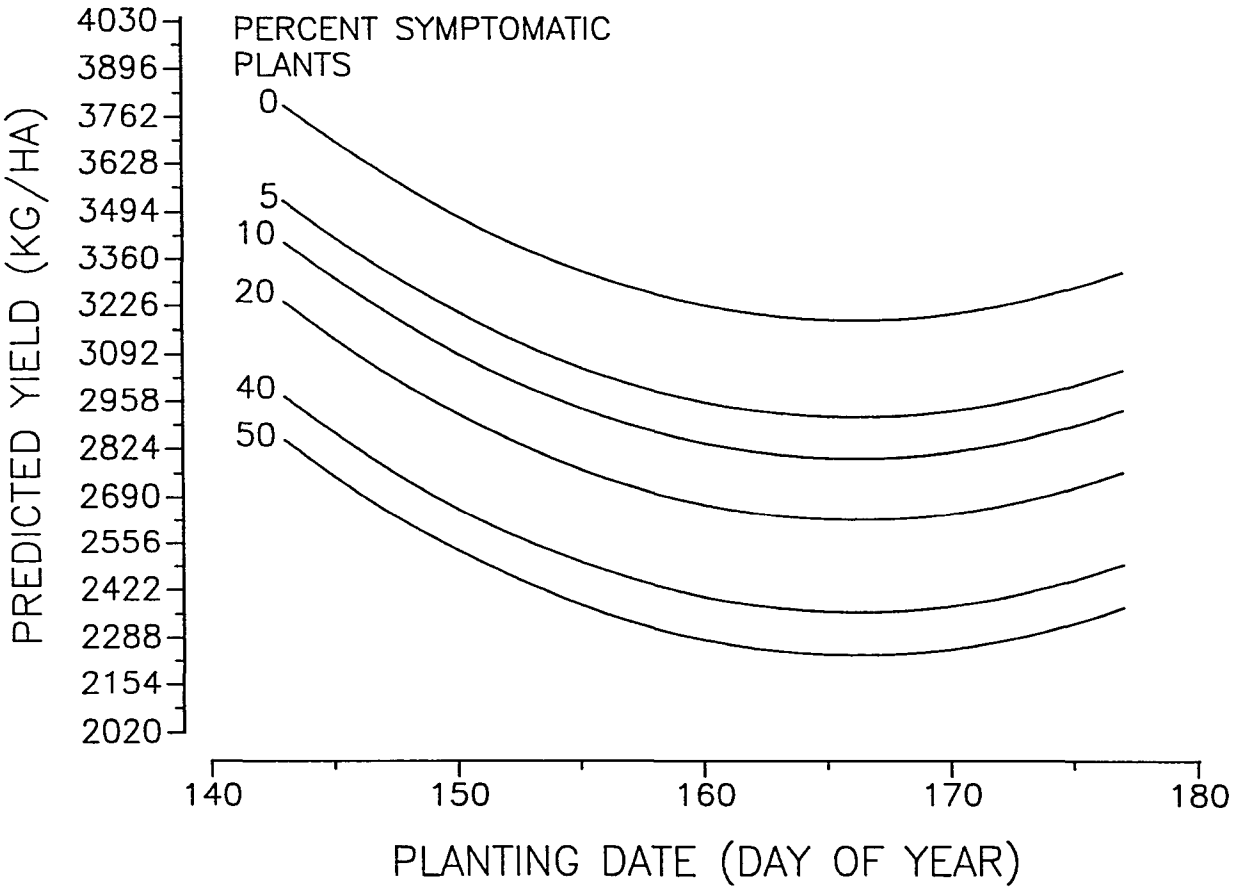

Fig. 1.6. Predicted yields for the cultivar 'Bedford' at varying percentages of red crown rot incidence.

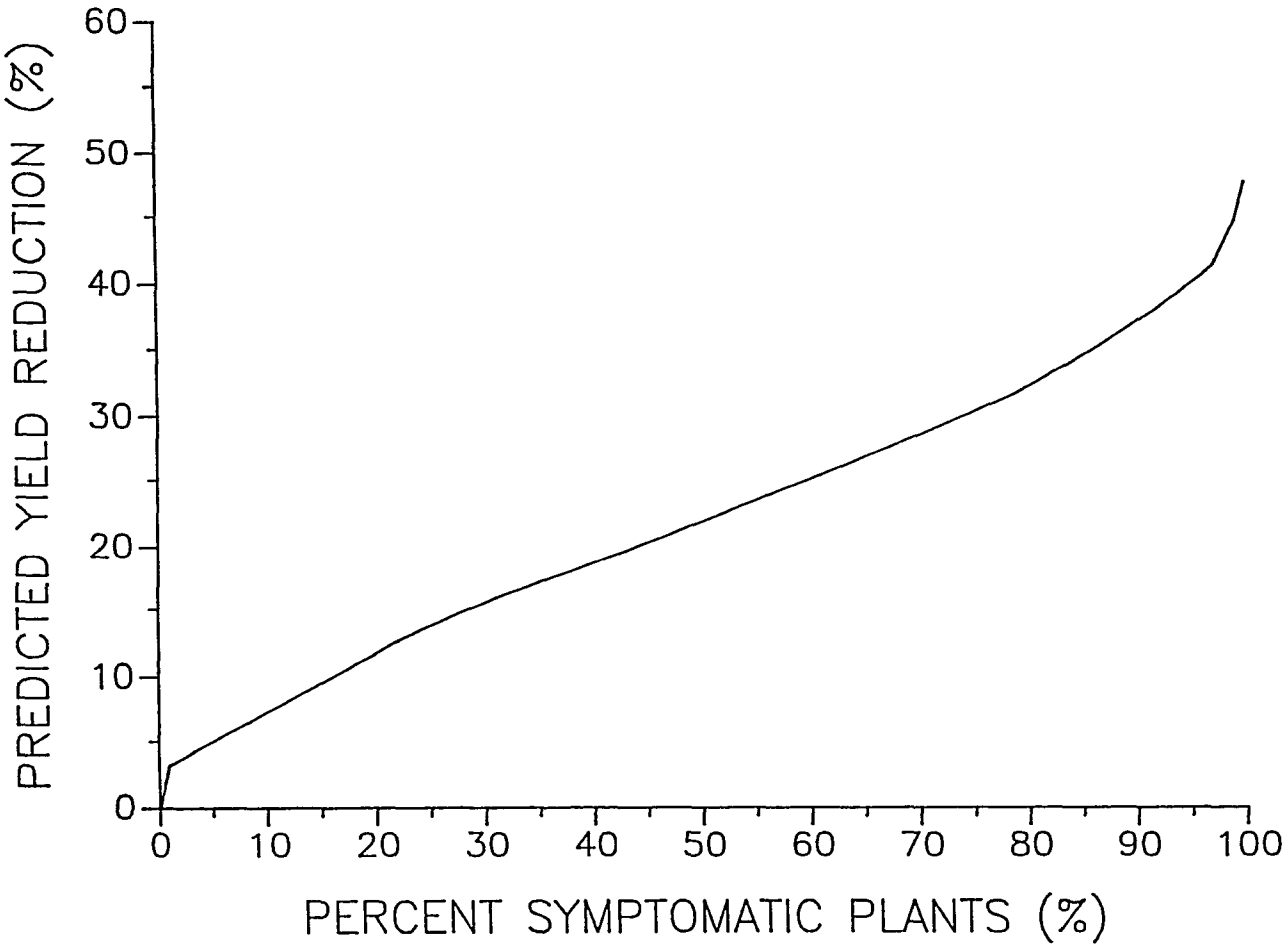

Fig. 1.7 Predicted yield reduction versus percentage red crown rot incidence for the cultivar 'Bedford' planted on day 143 (May 23).

21-74%, there was a buffered effect where the slope flattens out slightly. However, despite 100% incidence, the predicted yield loss was 50% or less which corresponds to estimates reported for severely-infested-fields (14).

SUMMARY

C. crotalariae, the causal organism of RCR, was probably introduced into Louisiana on indigo in the 1700's. From the indigo plantations, the organism most likely moved to surrounding areas by Mississippi River flood water which established the pattern of disease distribution in the state. On a smaller scale, surface runoff water probably plays a major role in the in-field distribution of the disease.

Disease assessment is difficult because of the late onset of symptoms and because of erratic canopy symptomology. The production of sexual fruiting bodies i.e., perithecia, at the base of the infected soybean plant is currently the best criterion for assessing disease. Based upon this assessment method, the maturity group VII cultivars generally appear to exhibit more resistance to the disease than maturity groups V and VI.

Delayed planting generally reduces RCR incidence, and this effect is most pronounced among susceptible cultivars. Yields of a resistant maturity group VII cultivar were not affected by RCR, and the optimum planting date was approximately June 2. Yields of a susceptible group V cultivar were significantly affected by RCR and planting date. However, the optimum planting date was independent of RCR pressure, and, within the range of this study, was May 23. By varying percentages of RCR within the framework of a statistical model, percentage yield loss for the susceptible cultivar was predicted. The most pronounced effects

were from 0-to-20% and 75-to-100% RCR incidence. At 100% evident infection, there was a predicted 50% yield loss.

LITERATURE CITED

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CHAPTER II

Effects of Glyphosate on *Calonectria crotalariae* and Red Crown Rot of Soybean

ABSTRACT

Surfactant and non-surfactant containing commercial herbicide formulations of glyphosate were evaluated for *in vitro* and *in vivo* effects on *Calonectria crotalariae* and red crown rot of soybean, respectively. Rates of 0.28, 0.56, 1.12, and 2.25 kg glyphosate/ha, corresponding to recommended rates for weed control in soybean (*Glycine max*) were used. Three pathogenic isolates of the fungus from soybean were grown on a selective medium amended either with water or different rates of the herbicide. Both formulations of glyphosate inhibited mycelial growth by *C. crotalariae*. Additions of amino acids to medium amended with the non-surfactant formulation produced a reversal of herbicide inhibition. Red crown rot incidence was reduced in field trials with preplant applications of low rates of glyphosate. These results, coupled with findings on the significant influence of previous season disease incidence on current red crown rot levels, indicate that glyphosate may be used simultaneously and efficaciously as a pre-plant herbicide for weed control and as a fungicide for the control of diseases caused by *C. crotalariae*.

INTRODUCTION

Calonectria crotalariae (Loos) Bell and Sobers (imperfect stage = Cylindrocladium crotalariae (Loos) Bell and Sobers) (3) causes the diseases Cylindrocladium black rot (CBR) and red crown rot (RCR) on peanuts (Arachis hypogea L.) and soybeans (Glycine max (L.) Merr.), respectively (4,12). Losses due to CBR on peanuts have been estimated to be as high as 53% (10) and a 50% yield loss can be expected on susceptible soybeans with 100% RCR incidence (4). The fungus overwinters in soil and crop debris as small (35 - 425 μm diameter), irregularly-shaped microsclerotia, that function as initial inoculum (11,13). The role of ascospores and conidia in the disease cycle is unknown although they may serve to colonize soil organic matter (8). Control measures for both diseases include the use of resistant varieties (2,4, 16) and practices directed toward avoiding or reducing initial inoculum such as delayed planting (4,15) and, in the case of peanuts, soil fumigation (2). Because of the expense of soil fumigation, the use of fumigation is the basis of an economic decision making model for peanut growers (2). Fungicides that can economically control RCR have not been identified.

In RCR field studies conducted between 1986 and 1988, glyphosate-containing herbicides were used for preplant weed control. The field studies have relied on naturally-occurring inoculum of C. crotalariae and, over time, a noticeable association between weed numbers and incidence of RCR developed. Weed-free areas were disease-free while weed-infested areas had high RCR incidence. Glyphosate-containing herbicides have some fungicidal activity (5,6,9,14), but often the fungicidal

activity is attributed to the surfactant contained in some of these herbicides (5,14).

The biosynthesis of phenylalanine, tyrosine, and tryptophan in plants is blocked by glyphosate which inhibits the conversion of shikimate to chorismate (a precursor of these amino acids) (1). Additions of these amino acids to plant tissue culture media has reversed the effects of glyphosate (7). If fungitoxic effects of glyphosate formulations on C. crotalariae could be affected similarly, this would suggest that glyphosate per-se is responsible for the fungitoxic activity.

From past field studies, a significant positive relationship was observed between previous and current season RCR incidence (Berner, unpublished data). If preplant applications of commercial glyphosate formulations could be shown to reduce levels of RCR within a growing season then a novel and economical usage for the herbicide would be to provide preplant weed control while simultaneously and cumulatively reducing levels of RCR.

The first objective of this study was to determine if recommended herbicidal rates of commercial formulations of glyphosate would inhibit growth of C. crotalariae in vitro. Further objectives were: to determine what rates were most fungitoxic and whether isolates of C. crotalariae were affected similarly; to determine if glyphosate or some other ingredient in the commercial formulations was the fungitoxic agent; to assess the field efficacy of different recommended rates of glyphosate on RCR incidence.

MATERIALS AND METHODS

In vitro

Fungal isolates. One pathogenic isolate of C. crotalariae was collected from soybean plants with RCR symptoms in each of three areas in Louisiana (Maringouin, La. = MSES isolate, Burnside, La. = BS isolate, and St. James, La.= STJ isolate), and isolated on Phipps' semi-selective medium (11). Hyphal tips from each isolate were transferred to corn meal agar amended with 2% (v/v) glycerol. One isolate (MSES) was chosen to conduct the initial herbicide screening. The other two isolates were later tested against the 2.25 kg/ha rate of glyphosate.

Culture medium. The medium used for the herbicide studies was Phipps' semi-selective medium (11) without thiabendazole. Aliquots (100 ml) of liquified medium were measured into 250 ml Erlenmeyer flasks. The medium was then autoclaved and allowed to cool to 45 C before adding amendments. Each amendment was added to the medium by sterile pipette. For each treatment, 10 Petri dishes, each containing 10 ml of amended medium, were prepared.

Treatments. Glyphosate at 0.56 kg/ha was considered a '1X' rate for these studies. This corresponds to 0.86 g glyphosate/l medium. Roundup (41.0% isopropylamine salt of glyphosate, Monsanto Company, Agricultural Products, St. Louis, Missouri), which contains surfactant, and Rodeo (53.8% isopropylamine salt of glyphosate, Monsanto Company, Agricultural Products, St. Louis, Missouri), which does not contain surfactant, were evaluated. Amounts of herbicide needed to approximate field rates were calculated based on the surface area of the medium contained in a 90 mm diameter Petri dish. Aqueous stock solutions of the formulated herbicides were prepared so that rates

approximating 0.5X, 1X, 2X, and 4X could be tested by varying the amount of solution added to the medium. Final concentrations for 1X were 0.74 µl Roundup/ml medium and 0.60 µl Rodeo/ml medium. To compensate for possible dilution effects, control treatments were made by adding appropriate amounts of sterile distilled water, in lieu of herbicide, to the medium.

To simulate a repeat application of herbicide, 4 mm diameter mycelial plugs from treated 4-wk-old cultures of C. crotalariae were transferred to fresh identically-amended medium. The medium was prepared as described and ten Petri dishes were prepared for each treatment. This procedure was done sequentially to form original (1st) and 2nd cycle cultures. All cultures were grown in room lighting at ambient temperature.

Aqueous stock solutions of DL-phenylalanine (PHE), DL-tyrosine (TYR), and DL-tryptophan (TRP) were made in 0.1 M concentrations. To determine if the herbicide effects could be reversed by additions of amino acids, 3 ml of these solutions were added to medium prepared at the 4X rate of 3.44 g glyphosate/l medium (2.25 kg glyphosate/ha). The amino acids were added singly and in all combinations. Treatments of 2 amino acids received 6 ml total amendment/100 ml medium (3 ml of each amino acid) and the treatment with all three amino acids received a total of 9 ml/100 ml medium. Controls were: herbicide + no amino acid (9 ml HOH), no herbicide (8 ml HOH) + each of the above amendments, no herbicide (8 ml HOH) + no amino acid (9 ml HOH). Preliminary tests using herbicides, antibiotics, and water, each filtered through 0.2-µm sterile syringe filters, produced the same results as unfiltered amend-

ments (Berner, unpublished data). This experiment was conducted with the MSES isolate and the surfactant-containing glyphosate formulation (Roundup), and, to test the effects of surfactant on fungal growth, the experiment was also conducted twice with the MSES isolate and the non-surfactant formulation (Rodeo).

Analyses. Maximum colony diameter was measured after 2 wk of fungal growth. Colony area was then calculated. The rate response experiment was conducted twice and analyzed as a randomized complete block design using trials as blocks. All other experiments were analyzed as completely randomized designs. Because response to individual rates was of interest, treatment means were plotted with their associated standard errors. For these results to be readily translateable with the results of the in vivo studies, rates are expressed as equivalent weights of glyphosate/ha.

In vivo

Locations. Two fields with a history of RCR were used in these studies. These fields were located in St. Gabriel and Burnside, Louisiana. The Burnside location had been used extensively in the past for RCR studies and the St. Gabriel location was known to have a high infestation of C. crotalariae.

Treatments. The commercial glyphosate formulation used in this study was Roundup. Three glyphosate treatments were application rates of 0.56, 1.12, and 2.25 kg glyphosate/ha. These rates correspond to the 1, 2, and 4X rates used in the in vitro experiments. Treatments were applied immediately prior to planting. After application, four 1 m x 12 m rows of the RCR-susceptible soybean cultivar "Centennial" were planted in each treatment plot. Each treatment was replicated 8 times

in each location in a completely randomized design. Because disease data was available from the previous season at Burnside, the previous season's disease ratings were used as a covariate at this location to adjust for plot differences in initial inoculum. In addition to the herbicide treatments, three control treatments were used to determine both differences directly attributable to the herbicide and those attributable to weed densities. The control plots received no preplant glyphosate but received either 0, 1, or 2 postemerge applications of fomesafen plus fluazifop-P-butyl. Each of these controls were replicated 8 times in each location. All glyphosate treatments received 2 postemerge applications of fomesafen plus fluazifop-P-butyl for weed control. A general preplant application of pendimethalin was used at the St. Gabriel location. No general preplant herbicide application was used at Burnside.

Data collection and analyses. RCR incidence in each plot was determined by counting plants bearing C. crotalariae perithecia out of the number of plants within a randomly-selected meter of row. Random selections were accomplished by generating random numbers which coincided with steps into the plot area. At this number of steps, the percentage RCR incidence was determined. This procedure was conducted 8 times for each plot and the individual samples were averaged to form a plot mean. These means were then analyzed by analysis of variance or covariance and least square means and standard errors were generated for each treatment. Yield was measured by harvesting the middle two rows of each plot, weighing the seed, adjusting the weights to 13% moisture content, and expressing as kg/ha. Analysis of variance was

conducted on individual plot yields.

RESULTS

In vitro. Colony areas of the control treatments did not significantly decrease in either cycle with increasing HOH additions (Fig. 2.1). In both cycles, glyphosate amendments greatly reduced colony area compared to that of the controls. In cycle 1, colony area was reduced 80% with the 4X (2.25 kg/ha) rate compared to the unamended control. In cycle 2 this reduction was approximately 60%. The effect of the herbicide was significant at the lowest rates included in each cycle. In cycle 2 the 0.5X rate was not included because the cultures were contaminated. The differences in the water controls between the two cycles were probably due to variation in ambient temperature at which the cultures were grown.

All three isolates treated with the 2.25 kg/ha rate of glyphosate as Roundup (Table 2.1) responded similarly. The smaller colonies of all isolates grown on the herbicide-amended medium appeared to be more darkly pigmented than colonies grown on the control medium (Fig. 2.2), and they also appeared to have produced fewer microsclerotia per unit area. Colony growth was not uniform across the herbicide-amended medium and resulted in colonies that had a branched appearance (Fig. 2.2).

Both surfactant- and non-surfactant-containing herbicides reduced colony area significantly (Tables 2.2, 2.3, and 2.4). Colonies grown on medium amended with the glyphosate formulation containing surfactant (Roundup) averaged 42% of the area of the water controls. Colonies grown on medium amended with the herbicide without surfactant (Rodeo) produced colonies that averaged of 55% and 22% of the controls in the two trials, respectively. With the exception of the second trial with

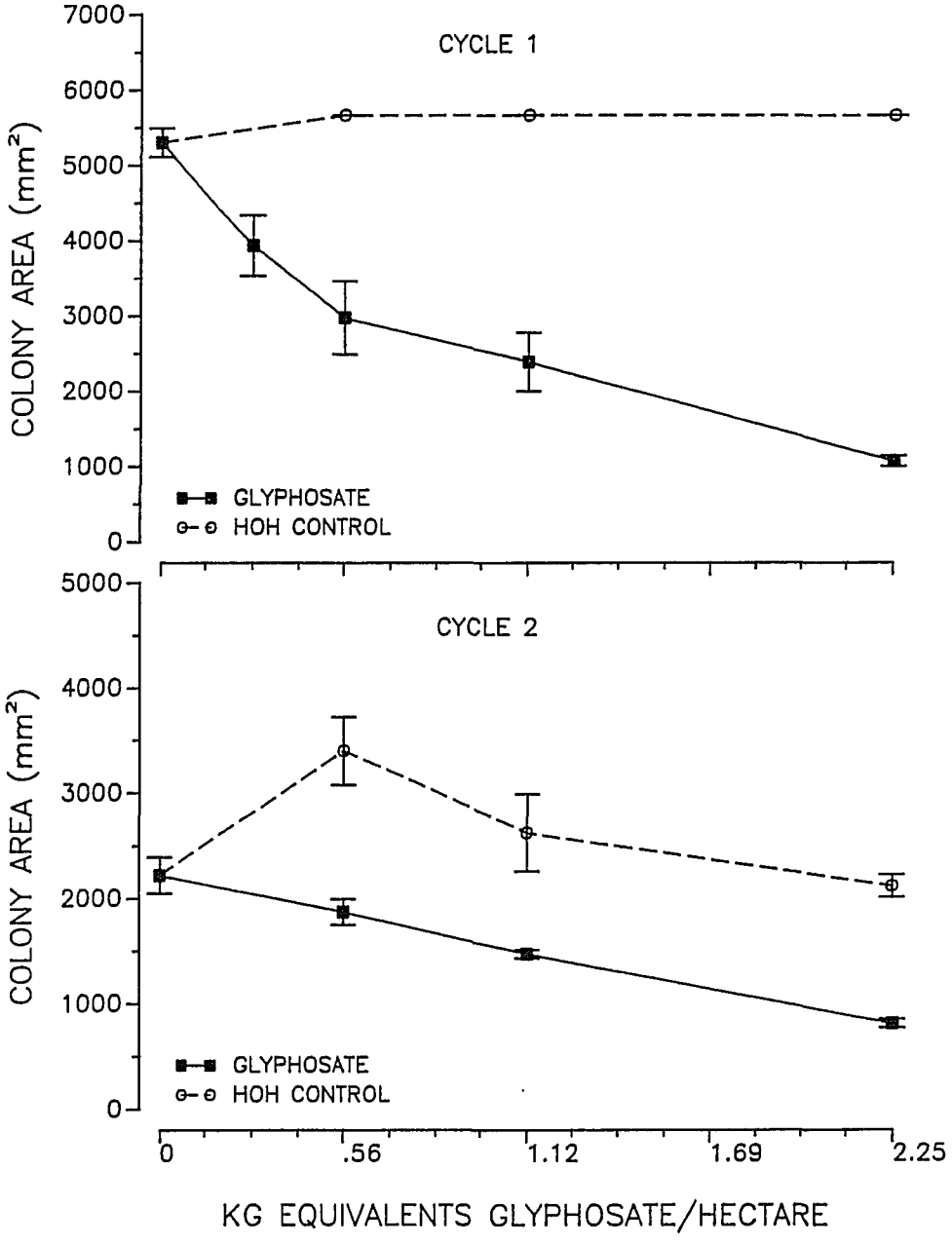

Fig. 2.1 Effects of glyphosate on C. crotalariae colony area after 2 wk growth. Data points are an average of measurements of colony diameter on medium in 10 Petri dishes/trial and two trials. Standard error bars are indicated. Standard error for cycle 1 HOH control = 0.0.

Table 2.1. Least square means of 2 week old colony area (mm^2) for three *C. crotalariae* isolates grown in media amended with either 8 ml stock herbicide solution (2.25 kg/ha glyphosate as Roundup) or 8 ml HOH per 100 ml medium.

	Medium amendment		
	Roundup	HOH	
Isolate			$LSD_{.05}$
MSES	926.9	2376.8	215.6
BS	735.0	1809.6	51.7
STJ	725.7	1566.5	184.6
$LSD_{.05}$[1]	93.2	355.9	
LSMEAN	795.9	1917.6	117.9

1/ LSD within columns based on harmonic mean of cell sizes.

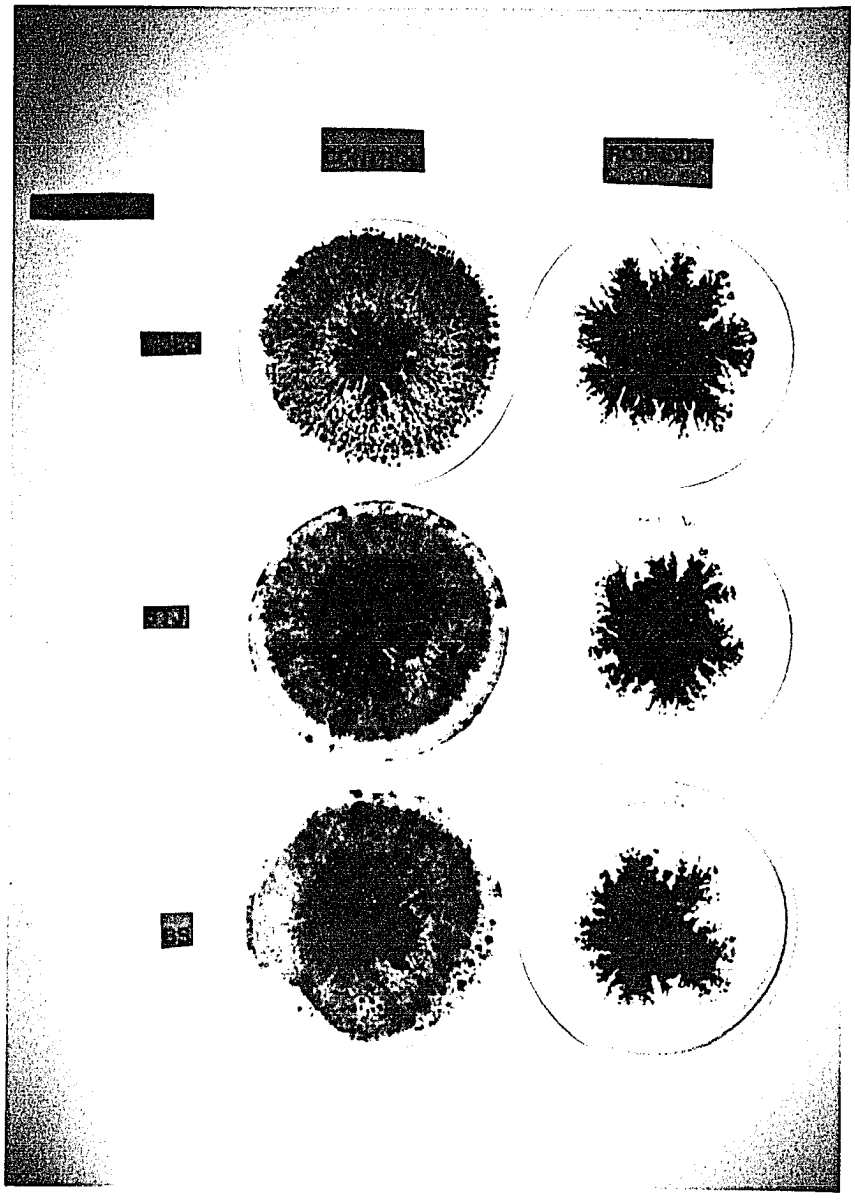

Fig. 2.2. Effects of glyphosate as Roundup on colony development of three isolates of C. crotalariae after 2 wks growth. Glyphosate rate is the equivalent of 2.25 kg glyphosate/ha. HOH controls contain 8 ml water/100 ml medium.

Table 2.2. Least square means of 2 week old colony area (mm^2) for *C. crotalariae* when treated with combinations of DL-phenylalanine (PHE), DL-tyrosine (TYR), DL-tryptophan (TRP) in media amended with either 8 ml stock herbicide solution (2.25 kg/ha glyphosate as Roundup) or 8 ml HOH per 100 ml medium.

	Medium amendment		
	Roundup	HOH	
Amino acid			$LSD_{.05}$
PHE	651.1	1505.8	275.9
TYR	851.8	1739.5	275.9
TRP	684.1	2282.8	275.9
PHE + TYR	455.6	1857.0	283.4
PHE + TRP	543.0	1185.3	275.9
TYR + TRP	883.7	1683.7	275.9
PHE + TYR + TRP	710.0	1685.5	318.6
None[1]	704.0	1124.3	283.4
$LSD_{.05}$ [2]	149.1	388.6	
Lsmean	685.4	1633.0	100.2

1/ Treatments with no amino acid received 8 ml of the respective amendment + 9 ml HOH.

2/ LSD within columns based on harmonic mean of cell sizes

Table 2.3. Least square means of 2 week old colony area (mm^2) for *C. crotalariae* when treated with combinations of DL-phenylalanine (PHE), DL-tyrosine (TYR), DL-tryptophan (TRP) in media amended with either 8 ml stock herbicide solution (2.25 kg/ha glyphosate as Rodeo) or 8 ml HOH per 100 ml medium -trial 1.

	Medium amendment		
	Rodeo	HOH	
Amino acid			$LSD_{.05}$
PHE	1781.9	3583.8	
TYR	1584.5	4027.8	
TRP	1880.6	3393.2	
PHE + TYR	1915.2	3831.0	352.0
PHE + TRP	1767.3	3531.8	
TYR + TRP	1976.8	3311.7	
PHE + TYR + TRP	2079.4	1886.5	
None[1]	1567.5	2844.6	
$LSD_{.05}$	154.1	479.6	
Lsmean	1819.2	3301.3	124.5

1/ Treatments with no amino acid received 8 ml of the respective amendment + 9 ml HOH.

Table 2.4. Least square means of 2 week old colony area (mm^2) for C. crotalariae when treated with combinations of DL-phenylalanine (PHE), DL-tyrosine (TYR), DL-tryptophan (TRP) in media amended with either 8 ml stock herbicide solution (2.25 kg/ha glyphosate as Rodeo) or 8 ml HOH per 100 ml medium - trial 2.

	Medium amendment		
	Rodeo	HOH	
Amino acid			$LSD_{.05}$
PHE	1377.3	5904.1	
TYR	849.2	5944.7	
TRP	952.1	5890.6	
PHE + TYR	1474.4	5944.7	
PHE + TRP	1910.5	5917.5	167.8
TYR + TRP	1022.6	5931.1	
PHE + TYR + TRP	1964.9	5472.4	
None[1]	953.5	5944.7	
$LSD_{.05}$ [2]	117.8	320.4	
Lsmean	1313.1	5868.7	83.9

1/ Treatments with no amino acid received 8 ml of the respective amendment + 9 ml HOH.

2/ LSD within columns based on harmonic mean of cell sizes

Rodeo, the additions of amino acids generally resulted in increased colony area for the water control treatments. In the second Rodeo trial, the colonies in most of the water control treatments had grown to the edge of the Petri dishes and enhancement of growth by amino acid additions was impossible to discern. In general, C. crotalariae growth responded favorably to the additions of the amino acids inhibited by glyphosate.

Additions of amino acids to Roundup-amended medium failed to result in colony sizes equal to the water controls, and, only in the case of the tyrosine plus tryptophan treatment, did the amino acid additions significantly increase colony area of the herbicide amended treatment over that of the control with no amino acid addition (Table 2.2). With the exception of the tyrosine treatment in the Rodeo amended medium in trial 1 (Table 2.3), all amino acid additions resulted in significantly larger colonies than the herbicide-amended medium with no amino acid addition. Fungal growth in the Rodeo-amended medium, containing additions of all three amino acids, did not differ significantly from the water control in trial 1. Growth in this treatment was also the greatest among all of the herbicide amended treatments. This seemed to indicate a reversal of the herbicidal effect, but, because the respective water control was significantly smaller than all other water controls, the experiment was repeated. In the second trial with Rodeo (Table 2.4), the addition of all three amino acids to the herbicide-amended medium, also resulted in the greatest fungal growth among the herbicide-amended treatments. Although fungal growth in all herbicide-amended treatments was significantly less than the water controls, the

herbicide treatment with the addition of all three amino acids resulted in radial growth over twice that of the herbicide treatment with no amino acid addition. With the exception of the tyrosine, tryptophan, and the tyrosine + tryptophan treatments all other amino acid treatments also resulted in significantly larger colonies than the herbicide treatment with no amino acid. Orthogonal contrasts were generated to compare the effects of each amino acid on the herbicide treatments. These contrasts showed that colonies in treatments containing phenylalanine and/or tryptophan were on the average significantly larger in both trials than the treatments containing tyrosine.

In vivo. There were no significant differences in RCR incidence among the three control treatments in either field location. Data from the three controls per location were pooled for subsequent analysis. Preplant applications of glyphosate as Roundup reduced RCR incidence at the 0.56 kg glyphosate/ha rate in both locations (Fig. 2.3). The reduction was substantial but non-significant at the St. Gabriel location, and higher application rates resulted in RCR incidences that were as great or greater than the controls. Regression analysis of two years of RCR incidence data from the Burnside location showed previous season disease incidence to be a positive and highly significant indicator of current season disease levels. When previous season disease incidence was used as a covariate to adjust initial disease levels at the Burnside location, a reduction in RCR incidence was seen at all preplant glyphosate application rates. This reduction was greatest at the 1.12 kg/ha rate where there was a clear separation of standard error bars, representing a significant difference at P=.16. When data from the two locations was combined (without benefit of the covariate) the

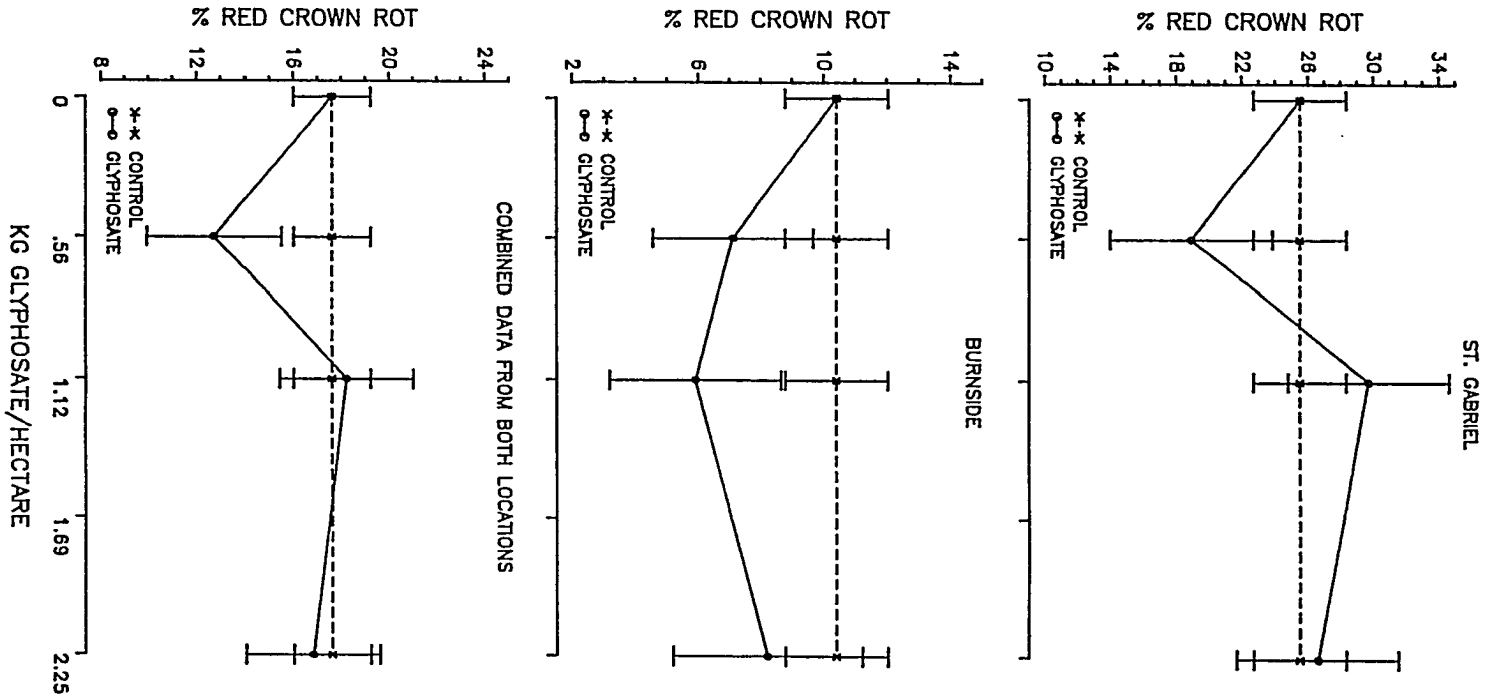

Fig. 2.3. Effects of preplant applications of glyphosate on RCR incidence in two field locations. Data points for each location are an average of 8 replications per glyphosate application rate. Data points for the combined data are an average of 16 replications. Control data points are an average of three control treatments per location and represent 24 replications per location and 48 replications for the combined data. Standard error bars are indicated.

greatest reduction in RCR incidence was seen at the 0.56 kg/ha rate, which was a significant reduction at P=0.13. The data combined from both locations also showed a trend toward higher disease incidence at the higher application rates. There were no significant differences in yield among any treatments at any location.

DISCUSSION

The glyphosate-containing herbicides used in this study greatly reduced growth of C. crotalariae in vitro and appeared to affect degree of pigmentation and microsclerotia development. These effects were universal for the isolates tested. The effect of increasing herbicide rates was additive, with the highest rates producing the smallest colonies. However, even low rates of herbicide reduced colony area significantly. These rates corresponded to field use rates of 0.28-0.56 kg glyphosate/ha, which are relatively low herbicide rates for preplant weed control in soybeans. Simulated repeat applications of herbicide did not enhance the fungitoxic effect.

The addition of amino acids, particularly phenylalanine and tryptophan, initiated a reversal or blockage of the herbicide effect in the experiments with Rodeo. This indicates that glyphosate per se is fungitoxic to C. crotalariae and that the biosynthesis pathway for these amino acids is probably the same in C. crotalariae as it is in plants. The failure of the amino acid additions to completely block the activity of the herbicide was probably due to dosage differences between the 3.44 g/l glyphosate concentration and the 0.1 M amino acid concentration. Because additions of amino acids did not reverse any herbicidal effect in Roundup treatments, the surfactant contained in this formula-

tion might have an additional fungitoxic effect that does not function in amino acid biosynthesis inhibition. We were unable to obtain a sample of the surfactant for direct testing.

The results of the in vivo studies are encouraging in two aspects. First, there was a direct reduction in disease incidence at the lowest rates in both locations which translated to a significant (P=0.13) reduction in RCR incidence for the combined data. Because there were no differences among the controls this reduction appears to be attributable directly to the fungitoxic effects of the herbicide rather than to any indirect role of weeds in RCR epidemiology. Although glyphosate is putatively degraded and/or immobilized by soil, it is translocated to the roots of target plants and may have an effect on soil microflora in the target plant rhizosphere (5). It may be by this mechanism that low preplant rates of glyphosate inhibit C. crotalariae and RCR development. At higher rates the herbicide may be less efficacious due to derogatory effects on soil microflora that are competitive with C. crotalariae.

Because there is a strong relationship between previous and current season RCR incidence, a second promising effect is cumulative reduction in RCR with preplant glyphosate applications being used over several seasons. The low rates at which glyphosate was effective present a novel and economic control strategy for C. crotalariae. Where RCR of soybean and CBR of peanut are major diseases, preplant weed control could be modified to include glyphosate. Glyphosate resistant cultivars would allow expansion of usage to pre- and post-emerge weed and disease control.

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CHAPTER III

A Rapid Procedure for Quantifying Microsclerotia from Cultures of *Calonectria crotalariae*

ABSTRACT

A rapid method for quantifying numbers of *Calonectria crotalariae* microsclerotia in culture was developed by blending solid medium cultures and analyzing the homogenate by colorimetry. Differences in microsclerotia concentrations were produced by adding different amounts of herbicides, known to have fungitoxic properties, to the culture medium. The colorimeter wavelength which produced the most reliable results was 900 nm. Coefficients of variation in absorbance of the homogenates at this wavelength were low, indicating the solid medium suspensions were stable enough to be analyzed by this procedure, and that 5-6 replications are suitable for detecting true differences of 25% of the mean. Liquid cultures were unsatisfactory for this procedure. The amount of time required to complete the procedure averaged approximately 7 min/sample which is dramatically quicker than making serial dilutions and replicated counts of microsclerotia.

INTRODUCTION

Red crown rot of soybean and Cylindrocladium black rot of peanut are two major diseases caused by the fungus Calonectria crotalariae (Loos) Bell and Sobers (2,4,5,8). The primary source of inoculum for the diseases is microsclerotia that overwinter in soil and crop debris, germinate in the spring, and infect susceptible plant roots (8). Microsclerotia vary in size from 35 - 425 μm in diameter (7) and are commonly quantitated from soil samples by a combination of sieving or elutriation followed by dilution, plating onto semi-selective media, counting colonies and calculating numbers per known volume or weight (5,7,8). Rapid screening of potential soil amendments that reduce initial inoculum requires in vitro assays of microsclerotia populations. These assays typically involve dilutions of homogenized cultures followed by either direct counts of microsclerotia or the above method. Both techniques are extremely time consuming. In this paper a colorimetric method to rapidly estimate the numbers of microsclerotia in culture media is reported.

MATERIALS AND METHODS

Sample preparation. Isolates of Calonectria crotalariae were grown on Phipp's medium (7), without thiabendazole in solid and liquid culture. Autoclaved medium was amended with amino acid biosynthesis inhibiting herbicides (imazaquin and chlorimuron ethyl) and sterile distilled water to form various chemical treatments and controls, respectively. The rates of imazaquin used in this study were 0.35, 0.69, 1.40, and 2.80 g/L of medium which correspond to 0.09, 0.18, 0.36, and 0.72 kg imazaquin/ha. The rates of chlorimuron ethyl used were 1, 2, 4, and 8 mg/L medium which correspond to 4.38, 8.75, 17.5, and 35 g chlorimur-

on ethyl/ha. Rates for both chemicals are approximately 0.5, 1, 2, and 4X recommended rates (1), respectively, for weed control in soybeans. To avoid dilution effects, a B-D Cornwall pipet (Becton, Dickinson and Company, Rutherford, N. J.) was used to measure 10 ml aliquots of medium into 50 ml test tubes (liquid culture) and 90 mm diameter disposable Petri dishes (solid culture). A 5 mm plug of fungus and medium was placed into each test tube and in the center of each Petri dish. The fungus was allowed to grow for 14 days on a lab bench at room temperature.

To analyze the differences in numbers of microsclerotia produced in these treatments, the fungus and medium in the test tubes were blended for 30 sec with a Sorvall Omni Mixer (Ivan Sorvall, Inc., Newtown, Connecticut) and decanted into colorimetry test tubes. The fungus and medium in Petri dishes were prepared by blending the contents of two dishes with 100 ml tap water in a Waring blendor (3 blend cycles of 10 sec each with intervals of 10 sec) and decanting into colorimetry test tubes. The tubes were inverted once every hour for three hours to mix the contents and bubble off trapped air. The contents were allowed to equilibrate overnight. The tubes were then inverted once, placed in a Spectronic 20 colorimeter (Bausch and Lomb, Rochester, N. Y.), and absorbance recorded.

Calibration and standardization. To determine optimum colorimeter wavelength, blanks were prepared, as described above, from liquid and solid media free of fungus and their absorbance was set at 0. The water-amended controls with the most apparent microsclerotia production were used to define the other end of the absorbance spectrum. The col-

orimeter wavelength that achieved maximum deflection between the blanks and the controls was used. Controls and herbicide treatments that resulted in obviously different levels of microsclerotia production were used in standardization. After blending the cultures, a subsample of 25 ml (roughly 1/5 of the whole sample) was diluted to 100 ml and a 1 ml subsample was placed on a small grided plastic dish. The dish was placed under a dissecting microscope and the microsclerotia were counted directly at 5X magnification. Microsclerotia concentrations per ml of culture homogenate were determined. Each colorimetry sample was counted four times and the mean concentration calculated. Each treatment and control was replicated four times and this procedure was repeated for each replication. Variation in absorbance at "high" and "low" microsclerotia concentrations was determined from 10 subsamples at each concentration. "Low" concentrations were set at 0-1000 microsclerotia/ml and "high" concentrations were set at >1000/ml. To reduce variation in the construction of a standard curve, means of the absorbances and overall mean concentrations were used. These data were fit to non-linear regression models and the model with the highest R^2 and least significant lack of fit was plotted as the standard curve.

RESULTS

Numerous microsclerotia were produced on water-amended medium after 14 d growth in both liquid and solid media. Cumulatively-reduced numbers of microsclerotia were formed with increasing herbicide rates on the medium amended with imazaquin. Liquid cultures were unsuitable for colorimetry for several reasons: 1) The fungus formed clumps of mycelia and microsclerotia that were difficult to uniformly homogenize; 2) The lack of agar resulted in an aqueous homogenate which allowed the

microsclerotia to rapidly settle out. This, in turn, produced erratic absorbance readings. Conversely, suspended agar in solid media homogenate kept microsclerotia in a uniform suspension for up to 3 h (after overnight equilibration).

The colorimeter wavelength which gave maximum differences between the blank and "high" microsclerotia concentrations for solid media samples was 900 nm. Coefficients of variation for absorbance, among samples at "high" and "low" microsclerotia concentrations, were 3.89% and 11.51%, respectively. Using the higher c.v. and tabulated values (6), the number of replications needed to detect a true difference of 305 microsclerotia/ml (25% of the mean) between treatments was between 5-6 at α=0.05 and 9-10 at α=0.01. Because two Petri dishes are used per replication, these numbers represent 10-12 and 18-20 Petri dishes, respectively, per treatment.

The standard curve prepared from data from solid media cultures is presented in Figure 3.1. The most sensitive portion of the curve is between absorbances of 0.20 and 0.50 and encompasses 80-90% of the microsclerotia concentrations observed in culture. The R^2 is significant at P=0.01 and the probability associated with lack of fit is 0.29.

DISCUSSION

The herbicides used in these studies were imazaquin and chlorimuron ethyl which have a fungitoxic effect on C. crotalariae and demonstrably reduce microsclerotia production at recommended herbicide rates (3). In order to efficiently quantify the effects of fungitoxic materials on the production of microsclerotia (the initial inoculum of C. crotalariae diseases) a rapid and objective technique was needed. From

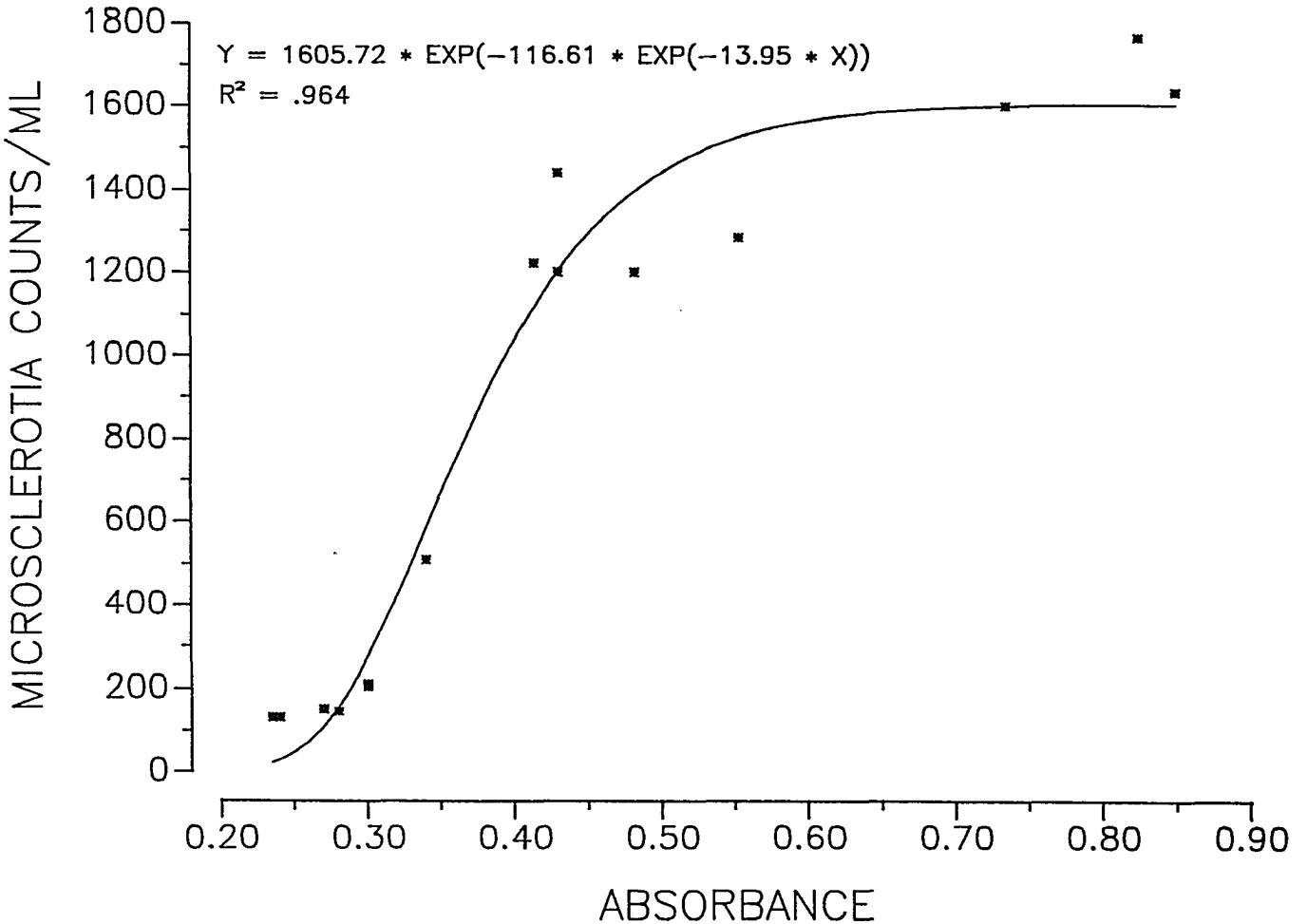

Fig. 3.1. Standard curve of microsclerotia counts/ml of homogenized medium vs. absorbance at 900 nm. Data points are means of 4 observations.

these studies, solid medium culture in combination with colorimetry proved to be the most desirable method for rapid quantification of in vitro microsclerotia concentrations. The low coefficients of variation indicate good stability of the solid media suspensions, whereas the greater amount of variation among samples of low concentration is probably the result of greater sensitivity of the colorimeter at low concentrations.

Serial dilutions of homogenized cultures followed by direct microsclerotia counts and colorimetry proved undesirable because aqueous medium dilutions resulted in absorbance differences independent of microsclerotia concentration. The use of incremental herbicide rate treatments to produce different microsclerotia concentrations produced a reliable standard curve that can be used with a high degree of accuracy, with adequate replication. To detect smaller differences, increased replication can be used (13 and 21 replications at α=0.05 and α=0.01, respectively, to detect a 183 microsclerotia/ml difference).

To use this procedure it is necessary to make blanks, "high" and "low" microsclerotia concentration standards, and calibrate the colorimeter before analyzing treatments. To make standards, Petri dishes from treatments which had obvious differences in microsclerotia concentrations were used. The concentrations were determined, after blending, by counting microsclerotia, and the "high" and "low" concentrations and the blank were used to calibrate the colorimeter to the standard curve. A problem with the system is the blank which is not accurate as a 0 microsclerotia/ml measure, since no mycelia are present (note the lack of a 0 point on the standard curve). However, the use of high and low standards as calibrating tools compensates for this deficiency if the

blank is considered only as a rough estimate of 0 and is used only as a calibration starting point.

Although some direct counting is necessary for calibration, this procedure is considerably faster than serially diluting and direct counting each sample (several times for accurate concentration estimates). For 40 samples (80 Petri dishes) a generous estimate of required time is 2 h for sample preparation, 1.5 h for counting and calibration, and 1 h for colorimeter readings. This averages 6.75 minutes/sample. Any treatment that induces differences in microsclerotia numbers can be readily assayed by this procedure.

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CHAPTER IV

Effects the Herbicides Imazaquin and Chlorimuron Ethyl, Alone and in Combination with Glyphosate, on Calonectria crotalariae and Red Crown Rot of Soybean.

ABSTRACT

The herbicides imazaquin and chlorimuron ethyl, registered for use in soybean, were evaluated in vitro for their potential to control C. crotalariae, either by inhibiting fungal growth or by directly reducing microsclerotia production. Isolates of C. crotalariae from soybean were grown on a minimal medium amended with commercial formulations of imazaquin and chlorimuron ethyl at 0.5, 1, 2, and 4X field use rates for weed control in soybeans. Each herbicide, at half rate, was also tested with low rates of glyphosate. Mycelia from each herbicide and rate treatment were transferred sequentially to identically-amended media to mimic repeated applications of the herbicides. Mimicked repeated applications of 2X rates of both imazaquin and chlorimuron ethyl significantly reduced colony area below that of the water control. Imazaquin significantly reduced microsclerotia production in each transfer cycle whereas the effects of chlorimuron ethyl were variable. Of the two materials, imazaquin appeared to be the most fungistatic. Additions of amino acids to imazaquin amended medium failed to reverse the herbicide effects. The lowest rates of imazaquin + glyphosate significantly reduced C. crotalariae colony size and these materials were further evaluated in field tests. No reduction in red crown rot (RCR) incidence was seen in field tests although cumulative microsclerotia reductions may gradually affect RCR incidence.

INTRODUCTION

Calonectria crotalariae (Loos) Bell and Sobers (imperfect stage = Cylindrocladium crotalariae) causes the diseases Cylindrocladium black rot (CBR) and red crown rot (RCR), on peanuts and soybeans, respectively (2,5,12). In the eastern United States CBR has long been a major disease of peanuts (2,12) and, in Louisiana, reports of RCR have been increasing since the early 1980's. RCR is now regarded as one of the more serious soybean diseases in Louisiana (5) and is becoming more important in Mississippi (J. P. Damicone, personal communication). Yield loss estimates range from highs of 53% for CBR on peanuts (1,8) to 50% for RCR on soybeans (5). The fungus overwinters in soil and crop debris as small (35 - 425 µm diameter) irregularly -shaped microsclerotia which function as initial inoculum (10,12). Control measures for both diseases include the use of resistant varieties (5,7,18), and practices directed toward avoiding or reducing initial inoculum e. g. delayed planting (5,16), and, in the case of peanuts, soil fumigation (9). Because the pathogen is soilborne, inexpensive chemical control measures are unavailable.

In field research with RCR a positive correlation between weed infestation and disease incidence was seen where amino acid biosynthesis-inhibiting herbicides were used (3,4). Recently, the fungitoxic effects of glyphosate on C. crotalariae was discovered (3). At recommended field rates for weed control in soybean, glyphosate significantly reduced C. crotalariae colony area in vitro and, at low rates, significantly reduced RCR incidence in the field. In the field studies in which herbicidal effects on RCR incidence were initially observed, two

herbicides other than glyphosate, which are also amino acid biosynthesis inhibitors, had been used. These materials were: Scepter (American Cyanamid Company, Wayne, NJ) which contains 180 g/l of the active ingredient imazaquin and Classic (E. I. du Pont de Nemours & Co., Inc., Wilmington, DE) which contains 250 g/kg chlorimuron ethyl. Although different chemicals, the presumptive mode of herbicidal action of these materials is non-competitive inhibition of acetohydroxyacid synthase (acetolactate synthase) (6,14). Inhibition of this enzyme results in subsequent inhibition of valine, leucine, and isoleucine synthesis (6,14). Additions of these amino acids to culture media reversed herbicide effects in plant tissue culture (11). Selectivity is due to degradation of the active ingredients in tolerant plants (6,11, 14). Although some herbicides have fungitoxic activity (17,15), this activity has generally been observed at very high application rates.

The objectives of this study were: to determine if commercial herbicide formulations of imazaquin and chlorimuron ethyl, at rates approximating those used in the field, would reduce fungal growth and/or affect microsclerotia development in vitro; to determine what rates were most fungitoxic; to determine if different C. crotalariae isolates would be similarly affected; to determine if any observed herbicidal effects could be reversed by the addition of amino acids; to determine the in vitro effects of each of these materials in conjunction with a known fungitoxic herbicide, glyphosate; and to examine the effects of preplant field applications of the more promising herbicide, alone and in conjunction with glyphosate, on RCR incidence.

MATERIALS AND METHODS

In vitro

Fungal isolates. One isolate of C. crotalariae, from each of three areas of Louisiana, was collected from soybean plants exhibiting RCR symptoms, and isolated on Phipps' semi-selective medium (10). Hyphal tips from each isolate were transferred to and cultures maintained on corn meal agar amended with 2% (v/v) glycerol. These isolates were designated "MSES" from Maringouin, LA; "BS" from Burnside, LA; and "STJ" from St. James, LA. The MSES isolate was chosen to conduct the initial herbicide screening. The other two isolates were later tested against 4X rates of the herbicides.

Culture media. The medium used for the herbicide studies was Phipps' semi-selective medium (10) without thiabendazole. Aliquots of 100 ml of liquid medium were measured into 250 ml Erlenmeyer flasks. The medium was then autoclaved and allowed to cool to 45 C before adding amendments. Each amendment was added to the medium by sterile pipette. Each treatment was composed of 10 petri dishes each containing 10 ml of amended medium pipetted into the dishes using a B-D Cornwall pipet (Becton, Dickinson and Company, Rutherford, NJ).

Treatments. Rates of imazaquin and chlorimuron ethyl at 180 g/ha and 8.75 g/ha, respectively, were considered '1X' rates for each chemical. Herbicide amounts needed to approximate field rates were calculated based on the surface area of medium contained in a 90 mm diameter petri dish. Aqueous stock solutions of the formulated herbicides were prepared so that rates of 0.5X, 1X, 2X, and 4X could be formed by varying the amount of solution added to the medium. These rates correspond to 90, 180, 360, and 720 g imazaquin/ha and 4.38, 8.75, 17.50, and 35 g

chlorimuron ethyl/ha. Final concentrations for 0.5X were 0.33 µl Scepter/ml medium and 1.0 µg Classic/ml medium. To compensate for possible dilution effects, control treatments were made by adding appropriate amounts of sterile distilled water, in lieu of herbicide, to the medium.

To simulate repeated applications of herbicides, 4 mm diameter mycelial plugs from 4 wk old cultures of herbicide treatments were transferred to fresh medium containing the same herbicide and rate. Medium was prepared as stated and ten petri dishes were poured for each treatment. This procedure was done sequentially to form original, 2nd , and 3rd cycle cultures.

Aqueous stock solutions of DL-valine (VAL), DL-leucine (LEU), and DL-isoleucine (ILE) were made in 0.1 M concentrations. To medium amended to the 4X herbicide rate, 3 ml of these solutions were added singly or in combinations to form VAL, LEU, ILE, VAL+LEU, VAL+ILE, LEU+ILE, and VAL+LEU+ILE treatments. Treatments with 2 amino acids received 6 ml total amendment (3 ml of each amino acid) and the treatment with all three amino acids received a total of 9 ml. Controls were: herbicide + no amino acid (9 ml HOH), no herbicide (8 ml HOH) + each of the above amendments, no herbicide (8 ml HOH) + no amino acid (9 ml HOH). To determine if the herbicide effects could be reversed by amino acid additions, 5 mm diameter plugs of mycelia and agar from 3rd cycle cultures were transferred to the amino acid amended media.

Combined treatments of imazaquin and chlorimuron ethyl with glyphosate were also studied *in vitro*. Combined rates for each of the materials with glyphosate were half of the 0.5, 1, 2, and 4X rates used

singly plus glyphosate rates the equivalent of 140, 280, 560, and 1120 g glyphosate/ha, respectively. The combined treatments were also compared to glyphosate treatments of 280, 560, 1120, and 2250 g/ha.

Analyses. Maximum colony diameter in each petri dish was measured after 2 wks of fungal growth. Colony area was then calculated. The rate response experiments were conducted twice and analyzed as randomized complete block designs. Because there was no significant difference between trials the data were pooled and treatment means were plotted with their associated standard errors. Microsclerotia production in all treatments was measured by blending two petri dishes of 2 wk old cultures with 100 ml water and analyzing by colorimetry, according to the method of Berner (4), wherein numbers of microscerotia/ml of culture homogenate are determined from a standard curve. These concentrations were statistically analyzed. For the results of the rate response experiments on colony growth and microsclerotia production to agree with the results of the in vivo studies, all rates were expressed as equivalent amounts of active ingredient / ha.

In vivo

Locations. Two fields (located in St. Gabriel and Burnside, Louisiana) with a history of RCR were used in these studies. The Burnside location had been used extensively in the past for RCR studies and the St. Gabriel location had a high infestation of C. crotalariae.

Treatments. As a result of the in vitro studies, imazaquin alone or in combination with glyphosate appeared to be promising fungitoxic treatments and were further evaluated in these field studies. The commercial formulations of these materials used in these studies were Scepter (17.3% imazaquin, American Cyanamid Company, Wayne, NJ) and

Roundup (41.0% isopropylamine salt of glyphosate, Monsanto Company, Agricultural Products, St. Louis, Missouri). The three imazaquin rates used were 0.18, 0.36, and 0.72 kg/ha and rates for the combined treatments were 0.09, 0.18, and 0.72 kg imazaquin/ha plus 0.28, 0.56, and 1.12 kg glyphosate/ha. These rates correspond to 1, 2, and 4X rates used in the in vitro experiments. Treatments were applied immediately prior to planting. After application, four 1 m x 12 m rows of the RCR-susceptible soybean cultivar 'Centennial' were planted in each treatment plot. Each treatment was replicated 8 times at each location in a completely randomized design. Because disease data was available from the previous season at Burnside, the previous season's disease ratings were used as a covariate at this location to adjust for plot differences in initial inoculum. In addition to the herbicide treatments, three control treatments were used to determine differences directly attributable to herbicide treatments and differences attributable to weed densities. The control plots received no preplant imazaquin or imazaquin + glyphosate but received either 0, 1, or 2 postemergent applications of fomesafen plus fluazifop-P-butyl. Each control was replicated 8 times at each location. The imazaquin and imazaquin + glyphosate treatments received 2 postemerge applications of fomesafen plus fluazifop-P-butyl for weed control. A general preplant application of pendimethalin for grass control was used at the St. Gabriel location. No general preplant herbicide application was used at Burnside.

Data collection and analyses. RCR incidence in each plot was determined by counting plants bearing C. crotalariae perithecia out of all the plants within a randomly-selected linear meter of row (5).

Random selections were accomplished by generating random numbers which coincided with steps into the plot area. RCR incidence was determined by counting the plants bearing perithecia and all of the plants within 1 m of row at the within-plot location corresponding to the random steps. The percentage incidence at each within-plot location was calculated. This procedure was conducted 8 times for each plot and the individual samples were averaged to form a plot mean. These means were then analyzed by analysis of variance or covariance and least square means and standard errors were generated for each treatment. Yield was measured by harvesting the middle two rows of each plot with a small plot combine, weighing the seed, adjusting the weights to 13% moisture content, and expressing as kg/ha. Analysis of variance was carried out on plot yields.

RESULTS

In vitro. A summary of the effects of imazaquin and chlorimuron ethyl on colony size is presented in Fig. 4.1. Reduction in colony size due to either herbicide treatment was observed only in the third transfer cycle. In cycles 1 and 2 both herbicide treatments at low rates produced significantly larger colonies than the water controls. As application rate increased in these cycles, colony size generally decreased, although the herbicices did not produce significantly smaller colonies than the HOH controls. In cycle 3, significant reductions in colony size were evident at equivalents of 17.5 and 35.0 g chlorimuron ethyl/ha and at 360 and 720 g imazaquin/ha. Colony size for the HOH controls remained fairly constant in all cycles with some minor fluctuation in cycle 3. The effects of the highest imazaquin and chlorimuron ethyl rates on colony size of all three isolates in the original trans-

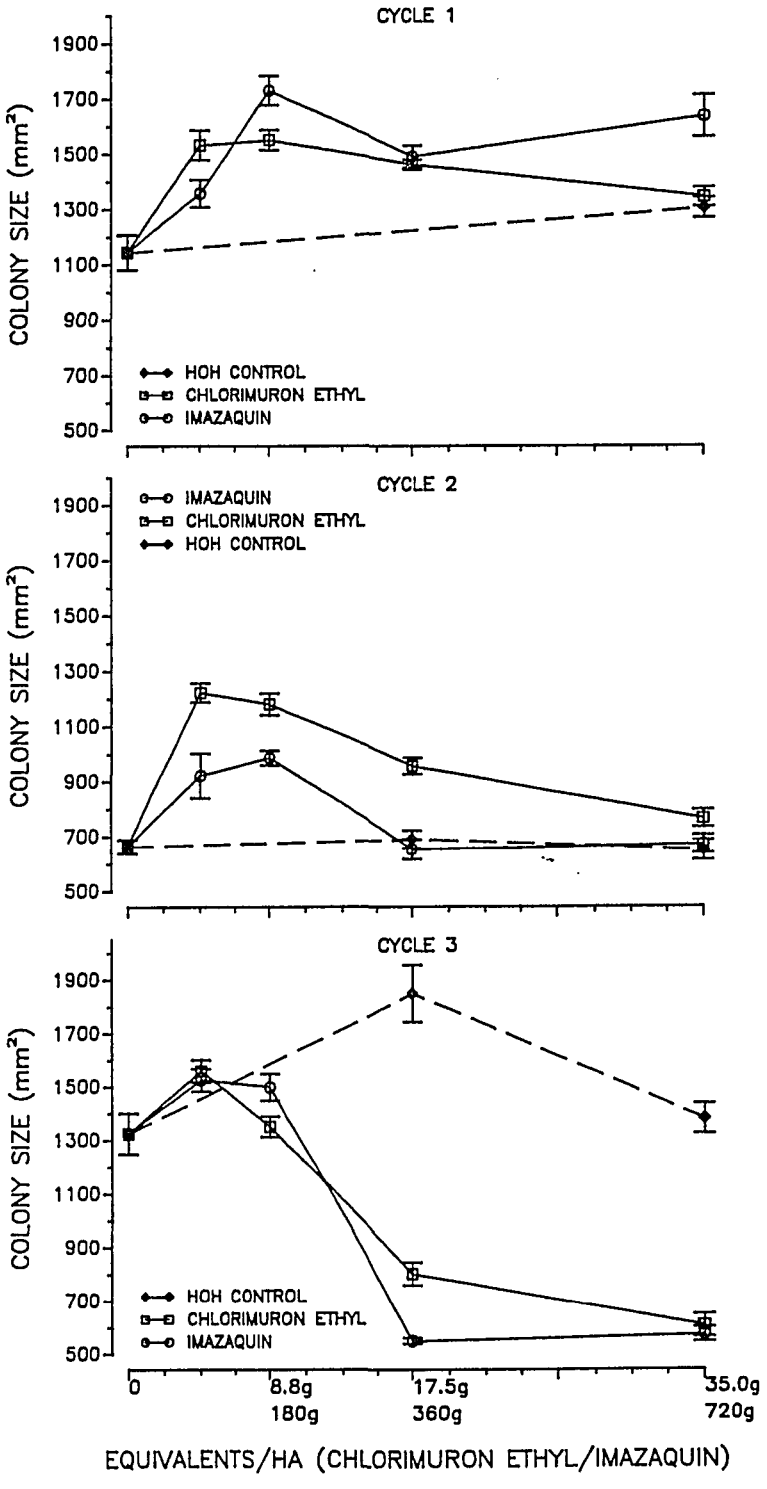

Fig. 4.1. Effects of chlorimuron ethyl and imazaquin and simulated repeat applications of these materials (cycles 1 - 3) on C. crotalariae colony area after 2 wks growth. Data points are an average of measurements of colony diameter on medium in 10 Petri dishes/trial and two trials. Standard error bars are indicated.

fer cycle is presented in Table 4.1. Chlorimuron ethyl did not produce colonies significantly smaller than the controls for any isolate. Colonies of STJ and BS isolates growing on either amended or control media were significantly smaller than colonies of the MSES isolate. The imazaquin treatment produced colonies significantly smaller than the water control with the BS isolate only. When comparing overall least square means (lsmeans), the imazaquin treatment resulted in colonies significantly smaller than the water controls while chlorimuron ethyl had no effect.

Microsclerotia production, as determined by colorimetry (4), was affected by imazaquin treatment (Fig. 4.2). Not only did this herbicide reduce total numbers of microsclerotia produced but it also encouraged production of larger sclerotia-like particles (Fig. 4.2). Total microsclerotia production was significantly reduced by imazaquin in all three transfer cycles (Fig. 4.3). The reduction was most pronounced at 360 and 720 g/ha rates but was also apparent at the 180 g/ha rate in cycle 1. Relative amounts of microsclerotia produced in the first culture cycle by all three isolates subjected to the 720 g/ha imazaquin rate are illustrated in Fig. 4.4. The effect of the herbicide on all isolates was primarily the reduction in amount of microsclerotia produced, but, in the case of the BS isolate, there was also a reduction in colony area similar to the effect observed with glyphosate (3).

Chlorimuron ethyl had variable effects on microsclerotia production (Fig 4.3). In cycle 1 there was no reduction due to this treatment at any rate. The 35.0 g/ha rate reduced microsclerotia production in cycle 2 but not in cycle 3. Conversely, the 17.5 g/ha rate resulted in

Table 4.1. Least square means of 2 wk old colony area (mm^2) for three *C. crotalariae* isolates in media amended with either 8 ml stock imazaquin solution (final concentration equivalent to 0.72 kg/ha imazaquin as Scepter) or 8 ml stock chlorimuron ethyl solution (final concentration equivalent to 35 g/ha chlorimuron ethyl as Classic) or 8 ml HOH per 100 ml medium.

	Medium amendment			
Isolate	Scepter	Classic	HOH	$LSD_{.05}$
MSES	2387.3	2671.5	2376.8	327.2
BS	898.5	1692.9	1809.6	373.9
STJ	1355.9	1728.8	1566.5	363.9
$LSD_{.05}$[1]	319.4	416.9	335.9	____
LSMEAN	1652.3	2112.6	1988.7	236.5

1/ LSD's based on harmonic mean of cell sizes.

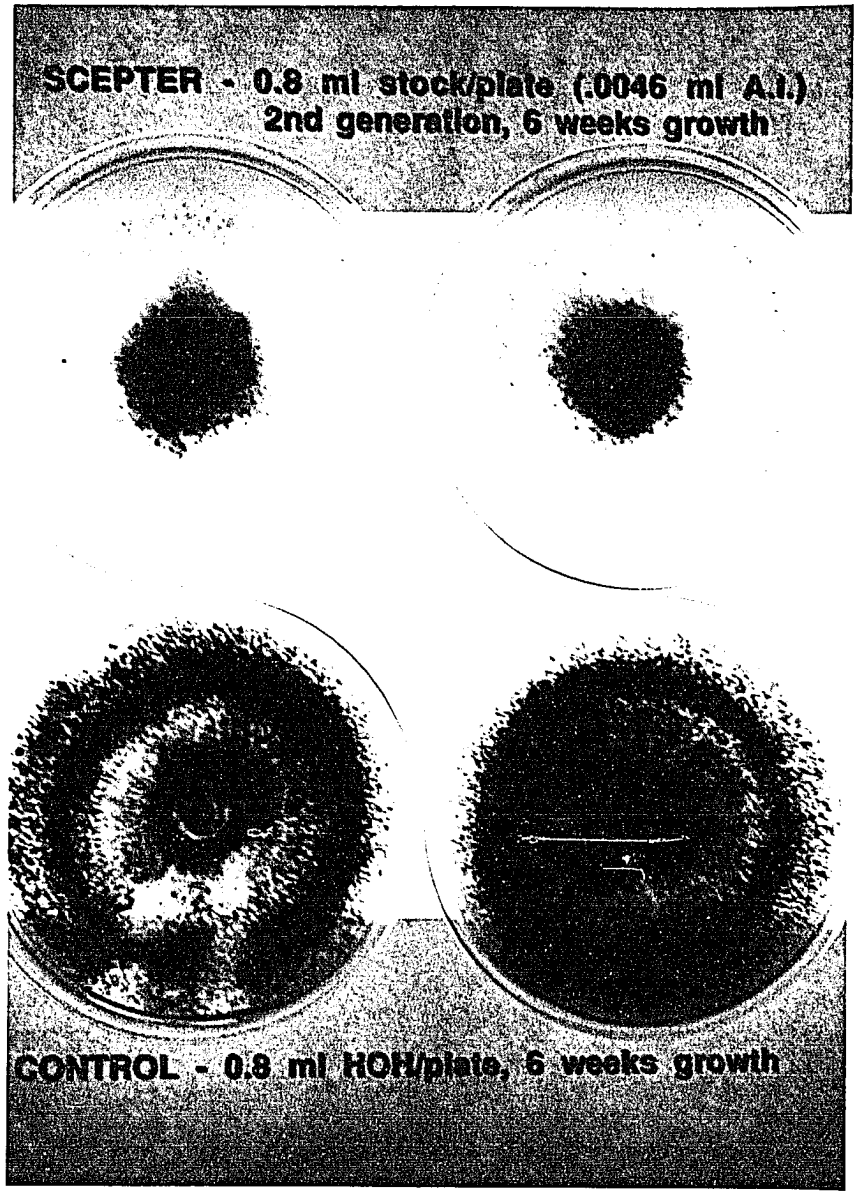

Fig. 4.2. Comparison of the effects of a medium amendment the equivalent of 0.72 kg/ha imazaquin as Scepter versus a water amendment on C. crotalariae colony development. The mycelia in all four Petri dishes have grown to the edge of the dish.

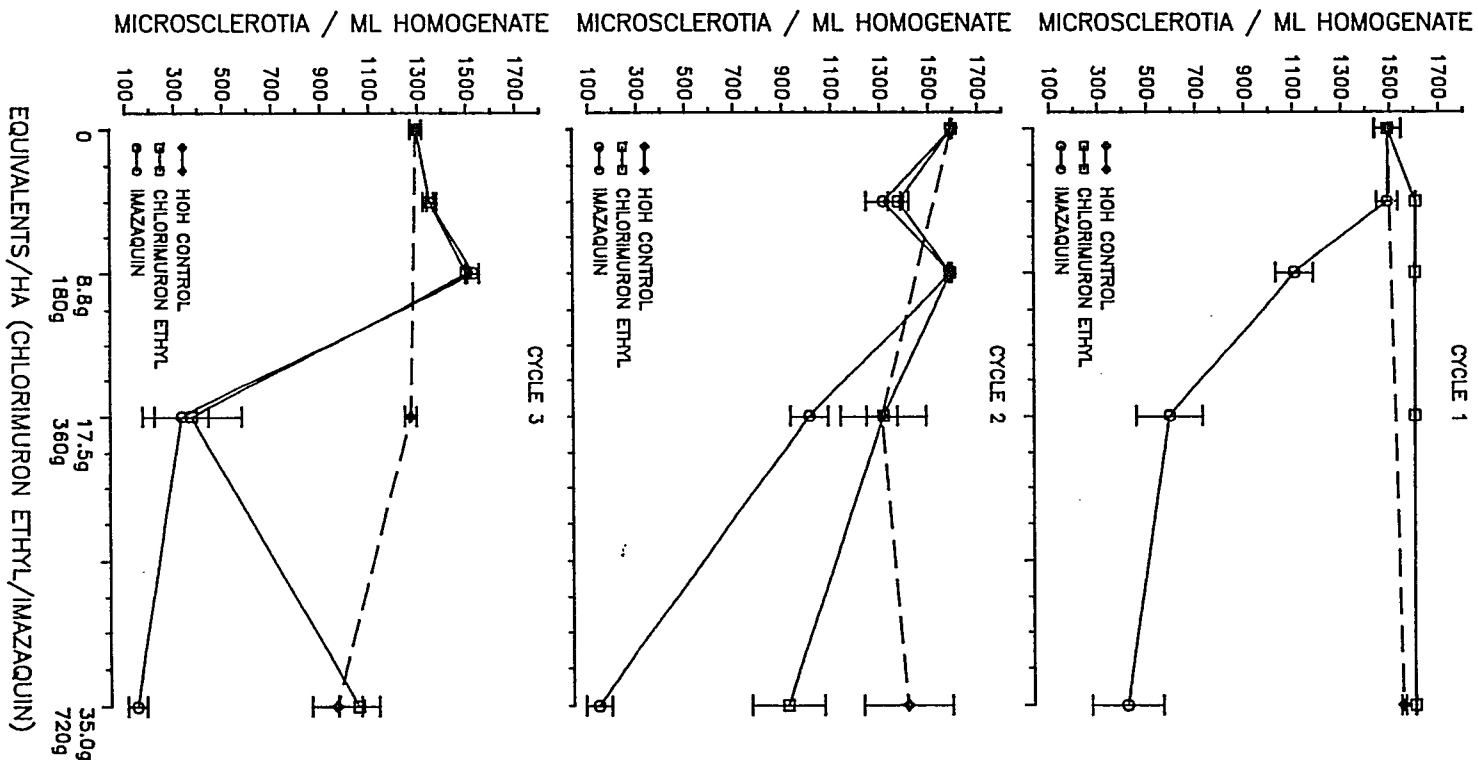

Fig. 4.3. Effects of chlorimuron ethyl and imazaquin and simulated repeat applications of these materials (cycle 1 - 3) on C. crotalariae microsclerotia production after 2 wks growth. Data points are an average of measurements on microsclerotia concentrations per ml C. crotalariae culture homogenate and reflect two trials of five replications each. Standard error bars are indicated.

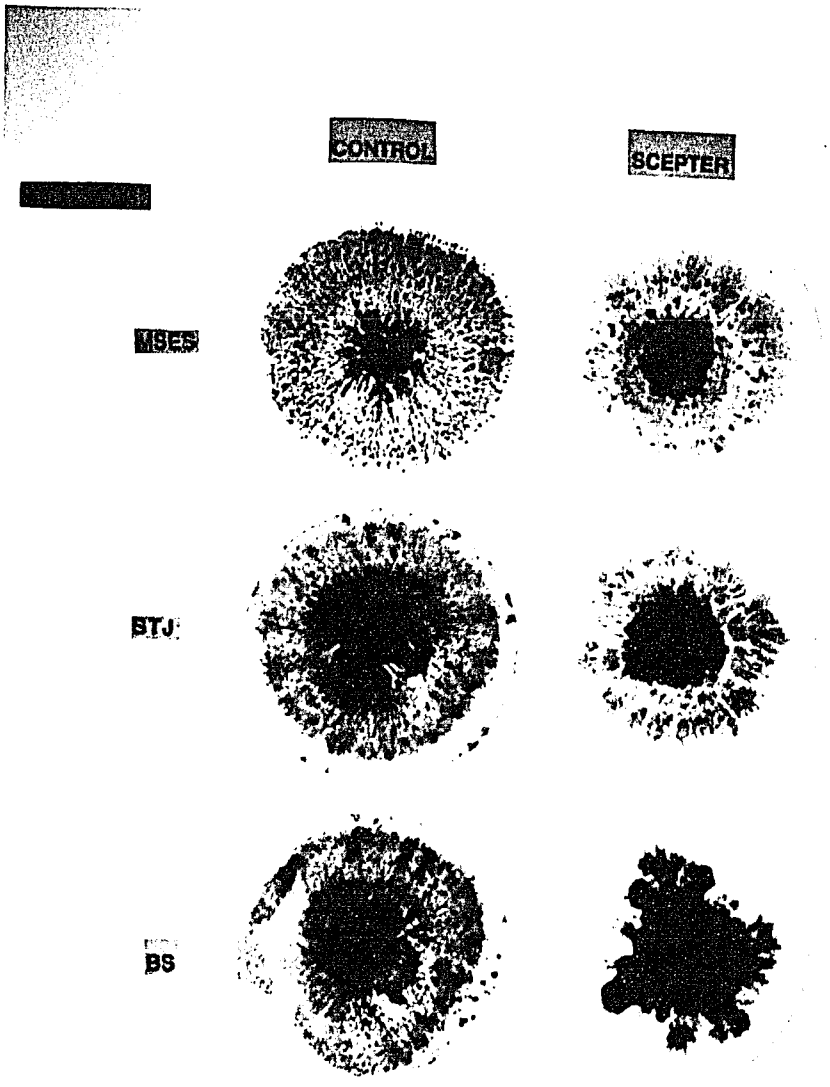

Fig. 4.4. Comparison of the effects of medium amendments the equivalent of 0.72 kg/ha imazaquin as Scepter versus water amendments on the colony development of three isolates of C. crotalariae. Colonies are 2 wks old.

fewer microsclerotia than the HOH control in cycle 3 but not in cycle 2. The highest herbicide rate produced no visible differences compared to the HOH controls for any of the isolates in the first culture cycle. Because of these variable and unpredictable effects, this material was not evaluated for any possible amino acid reversal of fungitoxicity.

Colony area of fungal isolates on imazaquin-amended medium differed from the control medium in a few cases when both were treated with the combinations of amino acids inhibited by this herbicide (Table 4.2). The overall lsmeans indicated that the imazaquin-treated medium produced significantly smaller colonies than the water controls. The effects of amino acid treatments on numbers of microsclerotia produced were highly variable (Table 4.3). The overall effect of imazaquin treatment was a significant and large reduction in microsclerotia below that of control treatments. In all amino acid treatments less microsclerotia were produced than the imazaquin-amended treatment in which no amino acids were added. In all but two treatments this difference was significant. The imazaquin treatment with all three amino acids produced the second fewest microsclerotia. Interestingly, the water control treatment that contained all three amino acids produced less microsclerotia than did any other water control. This difference was, however, not significantly less than the water control with no amino acid added.

When examined in combination with glyphosate, both imazaquin and cholorimuron ethyl significantly reduced colony area, at almost every application rate, in both transfer cycles (Fig. 4.5). This effect was most pronounced with the equivalent combined application of 280 g glyphosate + 90 g imazaquin/ha.

Table 4.2. Least square means of 2 wk old colony area (mm^2) for *C. crotalariae* when treated with combinations of DL-leucine (LEU), DL-isoleucine (ILE), DL-valine (VAL) in media amended with either 8 ml stock herbicide solution (final concentration equivalent to 0.72 kg/ha imazaquin as Scepter) or 8 ml HOH per 100 ml medium.

	Medium amendment		
	Scepter	HOH	
Amino acid			$LSD_{.05}$
ILE	332.3	347.9	74.2
LEU	355.0	370.1	77.6
VAL	337.6	360.5	82.4
ILE + LEU	319.0	428.8	54.4
ILE + VAL	375.1	349.1	76.6
LEU + VAL	341.3	400.4	63.8
ILE + LEU + VAL	269.0	400.4	139.7
None[1]	332.6	430.7	58.6
$LSD_{.05}$ [2]	68.3	75.0	
Lsmean	340.5	396.8	22.4

1/ Treatments with no amino acid received 8 ml of the respective amendment + 9 ml HOH.

2/ LSD within columns based on harmonic mean of cell sizes

Table 4.3. Least square means of numbers of microsclerotia / ml of 2 wk old C. crotalariae culture homogenate. Culture medium was amended with either 8 ml stock herbicide solution (final concentration equivalent to 0.72 kg/ha imazaquin as Scepter) or 8 ml HOH per 100 ml medium and then treated with combinations of DL-leucine (LEU), DL-isoleucine (ILE), DL-valine (VAL).

	Medium amendment		
	Scepter	HOH	
Amino acid			$LSD_{.05}$
ILE	1.9	1034.2	____
LEU	564.0	937.6	553.1
VAL	117.8	919.8	359.3
ILE + LEU	535.5	823.0	663.0
ILE + VAL	96.1	1092.8	128.1
LEU + VAL	67.8	1092.1	508.0
ILE + LEU + VAL	26.3	424.2	179.0
None[1]	1031.1	955.9	1189.3
$LSD_{.05}$ [2]	529.4	685.5	____
Lsmean	273.1	881.3	212.9

1/ Treatments with no amino acid received 8 ml of the respective amendment + 9 ml HOH.

2/ LSD within columns based on harmonic mean of cell sizes

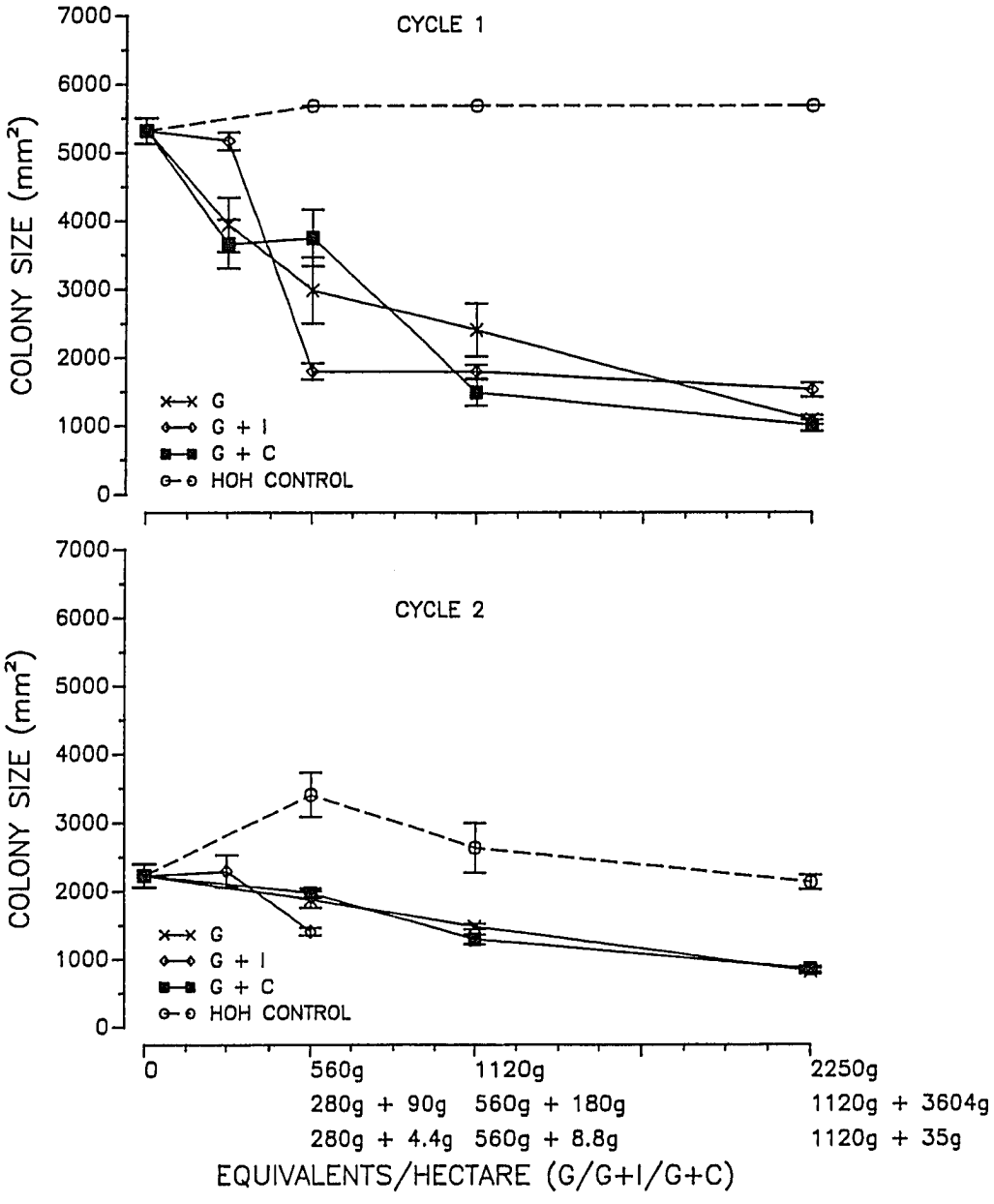

Fig. 4.5. The effects of three rates of glyphosate (G), glyphosate + imazaquin (G + I), and glyphosate + chlorimuron ethyl (G + C) and the effects of simulated repeat applications (cycles 1 and 2) on 2 wk old C. crotalariae colony size. Data points are an average of measurements on 10 Petri dishes/trial and two trials. Standard error bars are indicated.

In vivo. There were no significant differences in RCR incidence among the three control treatments at either field location. Data from the three controls per location were pooled for subsequent analysis. There were no significant reductions in RCR incidence attributable to either the imazaquin treatments (Fig. 4.6) or the imazaquin + glyphosate treatments (Fig. 4.7) at either location or for the combined data. There were no apparent consistent trends in the data for the imazaquin treatments, but there was an interesting effect of generally higher disease incidence at higher rates of combined herbicide treatments. In the case of the Burnside location (Fig. 4.7), there appears to be a slight reduction in % RCR at the lower rates of the combined treatments followed by an increase at the highest rate. The data in this graph were adjusted for the effect of previous season disease incidence which was shown to be a positive indicator of current season disease (3). There were no significant differences in yield among any treatment at any location.

DISCUSSION

Simulated repeat applications of both imazaquin and chlorimuron ethyl proved effective in reducing C. crotalariae colony area after the second transfer cycle. When the effects of these herbicides on colony area of three different isolates were examined, only imazaquin appeared efficacious. Imazaquin reduced microsclerotia numbers more than chlorimuron ethyl, independent of a simulated repeat application, and also qualitatively affected the size of microsclerotia produced (Fig. 4.2). Although chlorimuron ethyl seems to have some fungitoxic effects, it appears that either the fungus is less sensitive to it or that herb-

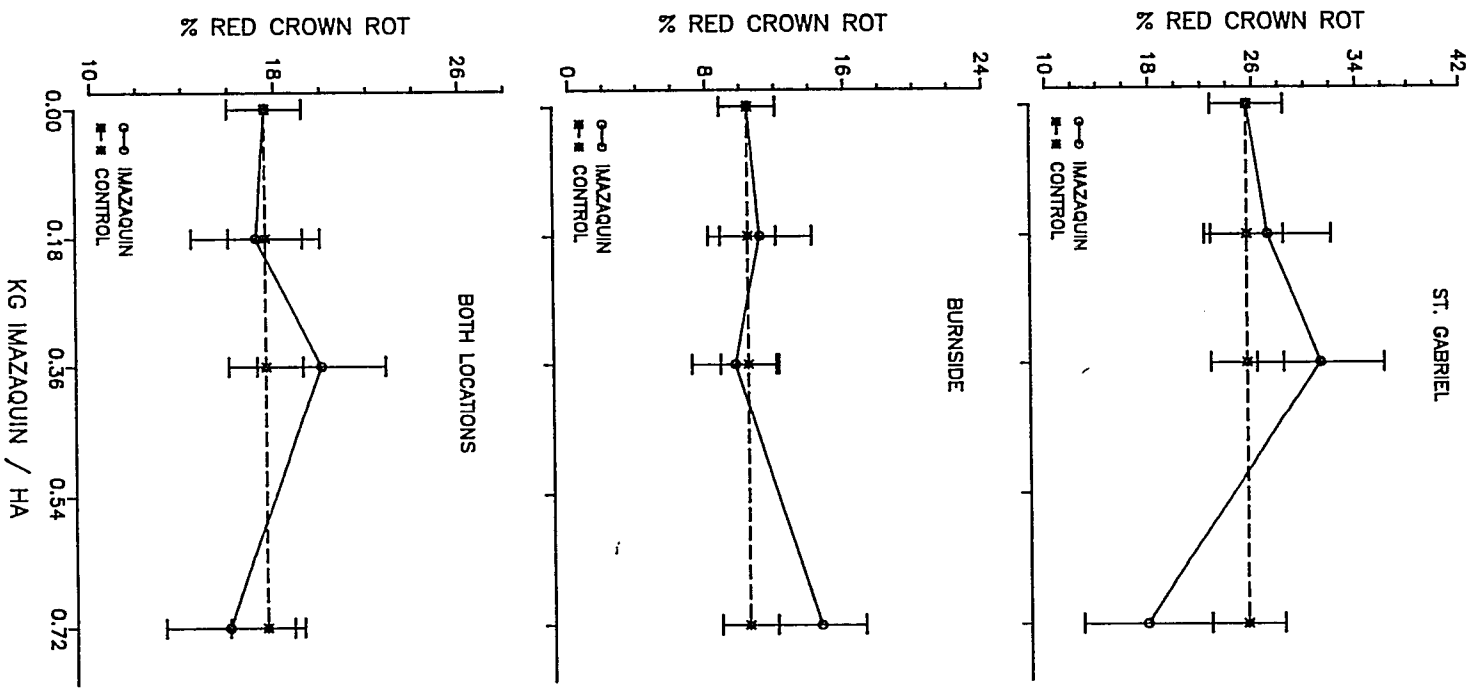

Fig. 4.6. The effects of three preplant application rates of imazaquin on red crown rot incidence in two locations. Data points for each location are an average of 8 replications per application rate. Data points for the combined data are an average of 16 replications. Control data points are an average of three control treatments per location and represent 24 replications per location and 48 replications for the combined data. Standard error bars are indicated.

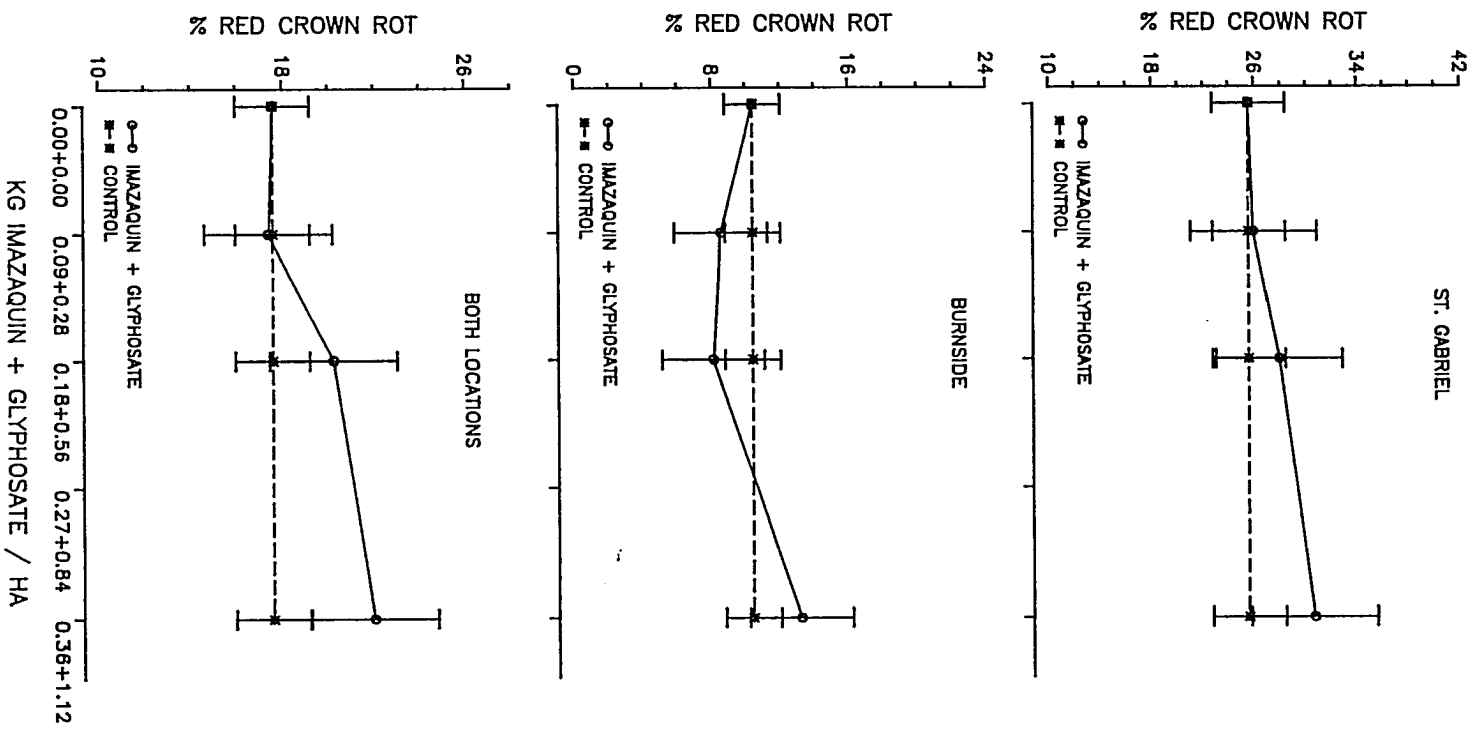

Fig. 4.7. The effects of three preplant application rates of imazaquin + glyphosate on red crown rot incidence in two locations. Data points for each location are an average of 8 replications per application rate. Data points for the combined data are an average of 16 replications. Control data points are an average of three control treatments per location and represent 24 replications per location and 48 replications for the combined data. Standard error bars are indicated.

icidal rates within the range of those recommended for soybean weed control are subcritical.

From these data and the known site of herbicidal acitivity of imazaquin (14) it seemed likely that the herbicide was interfering, either completely or partially, with the biosynthesis of leucine, isoleucine, and valine. However, when these amino acids were added to imazaquin-amended medium, no reversal or blockage of the fungitoxic effects were observed. There actually appeared to be an enhancement of fungitoxic effects with amino acid additions. There were minor differences among the amino acid amended and unamended treatments, but none were significant enough to indicate a reversal of fungitoxicity due to the addition of the respective amino acid(s). This phenomenon presents numerous aspects for speculation. Primary among these speculations is that imazaquin per se is not responsible for the fungitoxic effect but that an inert ingredient in the Scepter formulation might be responsible. Further speculations include differences in concentration between imazaquin and amino acid additions and the possibility that the biosynthesis of these amino acids follows an alternate pathway in C. crotalariae. Finally, the amino acids may not be getting into the fungus to change the effect. Regardless of how the effect is produced, the commercial formulation of imazaquin as Scepter appears quite fungitoxic to C. crotalariae in vitro.

When examined in combination with glyphosate, both imazaquin and chlorimuron ethyl, significantly reduced colony area more than the water controls. Since glyphosate dramatically reduces colony area (3) there is a corresponding indirect reduction in numbers of microsclerotia produced. Because of this complication, only colony area was anal-

yzed in the experiments with glyphosate. The most promising combination was that of the equivalent of 280 g glyphosate/ha + 90 g imazaquin/ha. These rates individually are approximately 1/2 the recommended rate of each of the materials for preemerge weed control in soybean. Because of the low rate, this combination presents an economic and novel possibility for the simultaneous use of these materials as herbicides and fungicides. The apparent efficacy of this combination led to the examination of these materials in field studies.

The failure of imazaquin treatments to reduce RCR incidence in the field was not surprising, since imazaquin was most effective in reducing numbers of microsclerotia, the initial inoculum, in newly formed colonies. The amount of inoculum present, prior to the experiment, would not be expected to be reduced by a preplant imazaquin treatment. Rather, a reduction in newly formed microsclerotia, functional as inoculum in the succeeding growing season, would be expected to be less. Because of this, additional field experimentation is needed to determine if there are possible cumulative effects of imazaquin on the reduction of RCR over time. Although these experiments were conducted with preemerge applications of imazaquin, Scepter is also a labeled and effective postemerge herbicide in soybean. Experiments on the effects of postemergent applications of imazaquin on RCR incidence are also warranted.

The field experiments with the combination of imazaquin + glyphosate are interesting because there is a great similarity in the data between these combined treatments and what was observed with preplant applications of glyphosate alone. In both locations and in the combined

data there is a trend toward increasing percentages of RCR at higher glyphosate application rates. This may be the result of the fungitoxic effects of glyphosate on soil microflora that compete with C. crotalariae.

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CHAPTER V

Soybean Resistance to Calonectria crotalariae

ABSTRACT

A procedure to evaluate soybean resistance to Calonectria crotalariae was developed and used to rate commercial cultivars for red crown rot (RCR) reaction. Cultivar trials were conducted for six years in a total of four locations throughout Louisiana. These trials showed consistent differences in levels of RCR incidence among cultivars. Location differences in RCR incidence for three cultivars showed a possible interaction which may be indicative of the existence of races of C. crotalariae. Apparent correlations between RCR incidence and cultivar maturity group were initially noted. This relationship was reevaluated on a yearly basis. Correlations between RCR incidence and yield were examined within each maturity group to determine if there were any differential effects on yield attributable to maturity. Data compiled from all trials showed a strong effect of within-row planting density on RCR incidence. This, in combination with other data, indicates at least two mechanisms of resistance.

INTRODUCTION

Red crown rot (RCR) of soybean (Glycine max (L.) Merrill) is caused by the fungus Calonectria crotalariae (Loos) Bell and Sobers (anamorph Cylindrocladium crotalariae) (1). The disease was first reported in Louisiana in 1976 in St. John the Baptist parish (2) and reports of RCR have increased steadily. Currently, there are 17 parishes that have confirmed reports of RCR (2). The most efficacious control measure is the use of resistant cultivars of which very few have been identified (2).

C. crotalariae overwinters in soil and crop debris as small (35 - 425 μm diameter) irregularly-shaped microsclerotia (8,10,14). The microsclerotia germinate in the spring and infect susceptible plant roots (10,14). Initial symptoms are interveinal chlorosis of the uppermost leaves followed by necrosis and shedding of the dead leaves. The distribution of the initial microsclerotia inoculum and the resultant disease is very aggregated (5,8) and symptoms do not appear until the R5 growth stage (4) or later (2). Following symptom development, infected plants will necrose and become obscured by the surrounding green, and apparently healthy, foliage (2). These aspects add to the difficulty in RCR assessment. Following the development of leaf symptoms, red perithecia are formed on the lower stem of infected plants. Ascospores are extruded from the perithecia in a viscous matrix and infrequently become airborne (12,16). Since they are formed late in the soybean growing season, they have no role in secondary disease development but are thought to function in the colonization of soil organic matter (9,10).

The fungus causes the disease Cylindrocladium black rot (CBR) (13)

in peanuts. The infection court has been reported to be the young hypocotyl region (7,11) indicating that infection takes place at an early stage. Our studies have indicated that infection in soybean can take place on roots late in the growing season and is not restricted to the hypocotyl (Berner, unpublished data). This large time span of infection, in combination with the other difficulties of RCR assessment, virtually precludes using RCR severity as an accurate measure of disease.

Peanut cultivar responses to C. crotalariae are quantitative, when measured as differences in disease incidence, and no immunity has been found (17). Levels of resistance in peanut appear to be related to root periderm thickness (7,11). Similarly, in soybean, there are gradations in RCR resistance although no immunity has been noted (2). From past studies (2), we have predicted soybean yield losses in susceptible cultivars to be as great as 50%.

The objectives of this study were to develop a method for evaluating cultivars for RCR reaction and then to use this procedure to identify resistant material. An additional objective was to examine RCR reaction in different environments and to see if there is any correlation between resistance and other characters and whether resistance is stable across environments.

MATERIALS AND METHODS

All cultivar trials were conducted in fields that had a history of red crown rot. Naturally-occurring inoculum was used in all trials. Trials were conducted for six years in multiple locations. These locations were: 1985 - Robert farm, Burnside, LA; 1986 - Robert farm,

Burnside, LA; 1987 - Robert farm, Burnside, LA, LaBoeuf farm, St. James, LA, Ward farm, Maringouin, LA; 1988 - Robert farm, Burnside, LA, LaBoeuf farm, St. James, LA, Ward farm Maringouin, LA; 1989 - Robert farm, Burnside, LA, Iberia Research Station, Jeanerette, LA; 1990 - Robert farm, Burnside, LA, Iberia Research Station, Jeanerette, LA, St. Gabriel Research Station, St. Gabriel, LA.

Two row plots x 6 m long were used for all cultivar entries except at the Iberia Research Station. Here the cultivar trials were four row plots x 12 m long. Between row spacings were approximately 1 m. In 1985, 1986, 1989, and 1990 four replications of each entry were evaluated in each respective locations. In 1987 and 1988 two replications per location were planted and rated for RCR. At the Iberia Research Station cultivars were planted in blocks according to maturity group. To facilitate timely harvest, the 1985 trials at the Robert farm were planted in two blocks corresponding to maturity groups V through VIII. Weed control was accomplished with commercial pre-and postemerge herbicides and cultivation.

A series of random numbers were generated that corresponded to steps into the plot area. At the specified random number of steps, a meter stick was laid next to the row and the plants were counted within that length of row. Out of this total, the plants bearing perithecia were also counted and percent incidence was calculated. Two of these counts were taken within each 6 m plot. RCR ratings from the two samples were averaged and the plot means were statistically analyzed. Because of the late onset of symptoms, plots were not rated until after the R5 growth stage. Cultivar means were calculated yearly and average

RCR ratings for maturity groups V, VI, and VII were calculated over the six-year period.

At maturity, each plot was harvested and seed weights were determined. Yields were adjusted to 13% moisture content and were expressed on a per hectare basis. To examine possible effects of maturity on RCR induced yield reductions, correlations between yield and RCR rating were calculated within maturity groups.

Because RCR incidence was calculated as a portion of the plants within 1 m row, within row planting density data was available. The large amount of data collected over the course of this study made it possible to compute an accurate correlation between planting density and RCR incidence. After computing this correlation we were able to make inferences not only about possible control measures but also about a possible mechanism of resistance.

RESULTS

Results of the trials are presented in Tables 5.1, 5.2, 5.3, 5.5, 5.7, 5.8, 5.9, and 5.10, for the years 1985, 1986, 1988, 1989, and 1990. Heavy rains during the early part of the 1987 growing season resulted in poor stands being produced at the Maringouin and St. James locations and no RCR data was collected. Poor RCR development at the Burnside location also precluded disease data collection in 1987. In 1985 differences in RCR incidence between maturity group blocks at the Robert farm were observed. Because of these differences, the results of each maturity group block are reported separately (Tables 5.1, 5.2). During the 1989 and 1990 growing seasons differences were observed between the maturity group blocks at the Iberia Research Station location. These blocks were also analyzed separately (Tables 5.7, 5.8,

Table 5.1. Red crown rot incidence and yield for maturity group V and VI cultivars, Robert farm, Burnside, LA - 1985.

Cultivar	Maturity Group	Red crown rot[1] Incidence (%)	Yield Kg/Ha
LA 79-813	VI	0.0	815.1
LANCER	VI	0.0	1879.4
DAVIS	VI	2.6	2485.7
TERRAVIG 505	V	2.6	2350.9
CENTENNIAL	VI	2.7	2896.6
TRACY M	VI	2.7	2452.0
DELTAPINE 506	VI	2.8	3031.4
TERRAVIG 606	VI	3.2	2647.4
LA 78-3208	VI	3.5	2101.7
LA 74-923	VI	3.6	1791.8
ASGRO 5474	V	4.5	2600.1
DELTAPINE 566	VI	4.7	2640.5
DELTAPINE 345	V	4.8	2465.4
BEDFORD	V	5.5	2404.7
RINGAROUND 680	VI	6.0	3051.4
COKER 156	VI	6.4	2943.6
LA 78-1732	VI	6.7	1347.2
WILSTAR 550	V	6.9	2290.2
PICKETT 71	VI	8.2	1178.8
DELTAPINE 105	V	8.4	2781.9
JEFF	V	9.1	2707.9
FORREST	V	10.2	2243.1
SHILOH	V	15.3	1879.3

Table 5.1. continued

Cultivar	Maturity Group	Red crown rot Incidence (%)	Yield Kg/Ha
HARTZ 6383	VI	15.4	2842.6
$LSD_{0.05}$		10.5	525.4

1/ Average percent of plants with perithecia from random plot samples of 1 m lengths of row.

Table 5.2. Red crown rot incidence and yield for maturity group VII and VIII cultivars, Robert farm, Burnside, LA - 1985.

Cultivar	Maturity Group	Red crown rot[1] Incidence (%)	Yield Kg/Ha
LA 79-11682	VII	0.0	2249.8
COKER 488	VIII	0.0	3051.4
WRIGHT	VII	0.4	2963.8
RANSOM	VII	0.5	2303.7
COBB	VIII	1.6	2707.9
COKER 237	VII	1.6	3064.9
COKER 338	VIII	2.1	2296.9
COKER 368	VIII	2.5	2923.4
TERRAVIG 808	VIII	4.0	1838.9
TERRAVIG 708	VII	5.2	2640.5
HARTZ 7126	VII	5.3	2977.3
WILSTAR 790	VII	5.7	3320.8
BRAXTON	VII	6.7	3213.1
LA 79-1004	VII	9.4	2519.3
BRAGG	VII	15.2	3132.2
LSD $_{.05}$		7.8	525.4

1/ Average percent of plants with perithecia from random plot samples of 1 m lengths of row.

Table 5.3. Red crown rot incidence and yield for maturity groups V-VIII cultivars, Robert farm, Burnside, LA - 1986.

Cultivar	Maturity Group	Red crown rot[1] Incidence (%)	Yield Kg/Ha
BRAXTON	VII	8.5	4277.4
TERRAVIG 808	VIII	9.9	2736.8
NKS 72-60	VII	12.9	2810.9
HARTZ 8112	VIII	15.1	3762.7
TERRAVIG 505	V	15.4	2767.1
WILSTAR 790	VII	15.4	3390.2
TRACY M	VI	17.2	3268.3
TERRAVIG 708	VII	17.3	3394.9
RANSOM	VII	18.6	3190.2
RINGAROUND 604	VI	18.9	2724.7
BEDFORD	V	20.0	2550.9
RINGAROUND 606	VI	20.5	3447.5
DELTAPINE 345	V	21.0	2901.2
ASGRO 7372	VII	21.5	3395.6
TERRAVIG 606	VI	22.3	3791.7
BRAGG	VII	22.5	2998.9
COKER 485	V	23.4	2772.5
RINGAROUND 680	VI	23.6	3377.4
WILSTAR 550	V	23.6	2629.7
DELTAPINE 105	V	24.1	3329.6
COKER 237	VII	24.3	3914.9
ASGRO 5474	V	24.9	2716.9
COKER 156	VI	25.2	3188.1

Table 5.3. continued

Cultivar	Maturity Group	Red crown rot Incidence (%)	Yield Kg/Ha
CENTENNIAL	VI	25.6	3003.5
YK 593	V	25.6	3178.0
YK 503	V	25.8	2143.4
NKS 69-54	VI	26.0	2957.8
DAVIS	VI	26.3	3345.8
DELTAPINE 506	VI	26.3	3332.9
WRIGHT	VII	27.7	3838.2
HARTZ 7126	VII	28.8	3225.9
ASGRO 5980	V	32.9	2975.3
YK 613	VI	34.1	2309.1
COKER 368	VIII	35.7	3516.2
ASGRO 6520	VI	36.3	2984.7
FORREST	V	37.1	2607.5
HARTZ 6383	VI	39.5	2701.5
HARTZ 5171	V	41.0	2722.7
LSD .05		16.5	1044.8

1/ Average percent of plants with perithecia from random plot samples of 1 m lengths of row.

Table 5.4. Average red crown rot incidence for 1985 and 1986 and yield averages for 1985, 1986, and 1987 for maturity groups V, VI, and VII.

Maturity Group	Red crown rot[1] Incidence (%)		Yield (Kg/Ha)[2]	
VII	9.4	A	3361.2	A
VI	16.8	B	2903.2	B
V	13.8	B	2465.4	C

1/ Average percent of plants with perithecia from random plot samples of 1 m lengths of row.

2/ Means followed by the same letter within a column are not significantly different (P=0.05)

Table 5.5. Red crown rot incidence and yield for maturity group V - VII cultivars, averaged over three locations (Robert farm, Burnside, LA, Ward farm, Maringouin, LA, and LaBoeuf farm, St. James, LA) in 1988.

Cultivar	Maturity Group	Red crown rot[1] Incidence (%)	Yield Kg/Ha
DELTAPINE 105	V	0.0	2923.4
ASGRO 6297	VI	4.3	2505.8
TERRAVIG 616	VI	4.4	3502.7
COKER 6847	VII	5.8	4041.6
VICTORY	VI	5.8	1764.8
NKS 72-60	VII	6.0	3300.6
DELTAPINE 566	VI	6.1	2957.1
HARTZ 5370	V	6.5	2943.6
ASGRO 7986	VII	6.9	5227.1
COKER 6727	VII	7.1	3098.6
DAVIS	VI	8.5	3731.7
BRAXTON	VII	9.1	3738.5
CENTENNIAL	VI	10.1	3240.0
HARTZ 6130	VI	10.4	2943.6
WILSTAR 550	V	11.0	2552.9
SAMPSON	VI	11.4	3745.2
TERRAVIG 708	VII	12.0	3098.6
YK 699	VI	12.0	2728.1
COKER 485	V	12.1	2903.2
TERRAVIG 505	V	12.6	2883.0
SHARKEY	VI	12.8	3954.0

Table 5.5. continued

Cultivar	Maturity Group	Red crown rot Incidence (%)	Yield Kg/Ha
BAY	V	13.5	2768.5
BUCKSHOT 703	VII	13.6	4014.7
FFR 561	V	15.3	2829.1
ASGRO 6785	VI	15.4	3704.8
TRACY M	VI	15.6	3314.1
BUCKSHOT 603	VI	15.9	3064.9
DELTAPINE 506	VI	17.1	3549.9
CAJUN	VI	17.4	3725.0
BEDFORD	V	17.4	1663.8
HARTZ 5171	V	17.9	3031.2
DELTAPINE 417	VII	19.0	3684.6
RINGAROUND 606	VI	19.4	3206.3
RINGAROUND 680	VI	19.5	3381.5
FORREST	V	19.9	2734.8
HARTZ 7126	VII	21.8	4034.9
ASGRO 5980	V	23.0	3112.0
HARTZ 5164	V	23.2	2896.5
HARTZ 6385	VI	24.4	3226.5
COKER 686	VI	25.0	3085.1
$LSD_{.05}$		16.8	1192.3

1/ Average percent of plants with perithecia from random plot samples of 1 m lengths of row.

Table 5.6. Average red crown rot incidence for 1985, 1986, and 1988 and yield averages for 1985-1988 for maturity groups V, VI, and VII.

Maturity Group	Red crown rot[1] Incidence (%)	Yield (Kg/Ha)[2]
VII	10.1 A	3583.6 A
VI	14.1 B	3064.9 B
V	15.1 B	2620.3 C

1/ Average percent of plants with perithecia from random plot samples of 1 m lengths of row.

2/ Means followed by the same letter within a column are not significantly different (P=0.05).

Table 5.7. Red crown rot incidence and yield for maturity group VI cultivars, Iberia Research Station, Jeanerette, LA - 1989.

Cultivar	Red crown rot[1] Incidence (%)	Yield Kg/Ha
RIVERSIDE 699	5.1	2108.4
HARTZ 6200	5.5	3260.2
RIVERSIDE 677	6.3	2303.7
DELTAPINE 726	7.1	2856.1
FFR 606	8.7	2081.4
DPX 3627	9.0	2815.6
PIONEER 9641	10.9	3307.4
LA 77-9729	11.1	2546.2
RINGAROUND 606	12.6	2829.1
LA 77-8715	12.8	2270.0
B2J	13.8	2768.5
TWIGGS	14.7	2755.0
EHJU 5	19.8	2445.3
BALDWIN	19.9	2047.9
LA 87-6002	20.2	2606.9
DAVIS	20.5	2175.8
BUCKSHOT 603	20.6	2633.9
ASGRO 6785	21.0	3240.2
LA 88-6028	21.4	2667.6
NKS 69-54	21.5	2573.3
HARTZ 6686	22.3	2586.8
TERRAVIG 626	22.3	2148.9
SHARKEY	23.0	3105.5

Table 5.7. continued

Cultivar	Red crown rot Incidence (%)	Yield Kg/Ha
FFR 646	23.3	2337.5
DELTAPINE 566	23.7	1926.6
FFR 695	23.7	2364.5
EHJU 11	23.9	2014.2
TERRAVIG 616	24.1	2485.7
SAMPSON	25.1	2425.0
RINGAROUND 680	26.1	2633.9
LA 87-6001	26.3	2728.2
RIVERSIDE 696	26.6	2371.2
AT-EXP-0695	26.9	2809.1
LLOYD	29.4	1872.7
CAJUN	29.7	3038.1
HARTZ 6570	30.2	2061.3
EXP-R2-604	30.6	2411.6
LA 88-6051	34.4	2445.3
LA 87-6013	35.7	2182.6
EHJU 7	36.1	1751.5
ASGRO 6297	36.3	2916.8
SPARTAN	37.0	2310.6
LA 87-6011	37.7	2169.1
DELTAPINE 506	38.0	2263.4
LA 87-6012	39.8	1980.5
PIONEER 9691	40.3	2364.5
LA 88-6055	40.3	2384.7

Table 5.7. continued

Cultivar	Red crown rot Incidence (%)	Yield Kg/Ha
LAMAR	40.9	2054.6
R1-11	41.9	2654.1
LA 88-6024	49.0	1960.3
DPX 3623	49.1	2263.4
CENTENNIAL	49.1	2121.9
LA 88-6010	49.6	2182.6
LEFLORE	49.9	1953.5
$LSD_{.05}$	24.8	613.0

1/ Average percent of plants with perithecia from random plot samples of 1 m lengths of row.

Table 5.8. Red crown rot incidence and yield for maturity group VII and VIII cultivars, Iberia Research Station, Jeanerette, LA - 1989.

Cultivar	Red crown rot[1] Incidence (%)	Yield Kg/Ha
HSC 721	1.8	3624.2
HARTZ 8112	10.3	3159.4
LA 79-11682	13.8	2809.1
ASGRO 7258	17.4	2633.9
HARTZ 7190	19.2	2822.5
RALLY	20.7	3132.4
HARTZ 7585	23.2	3166.1
LA 79-1133	23.7	2964.0
EHJU 3	26.0	2095.0
PIONEER 9791	26.0	2647.4
RIVERSIDE 757	26.5	2755.2
AGRATECH 700	26.9	2620.4
LA 87-7010	27.3	2553.1
LA 87-7032	27.5	2283.6
ASGRO 7986	30.3	2903.4
DELTAPINE 417	30.3	2249.9
LA 78-17908	31.5	2613.7
STONEWALL	31.8	2876.4
BRAXTON	32.5	2371.2
HARTZ 7126	33.6	2701.3
LA 87-7022	33.9	2398.1
LA 79-11123	34.5	2546.3

Table 5.8. continued

Cultivar	Red crown rot Incidence (%)	Yield Kg/Ha
BUCKSHOT 703	35.3	2640.7
LA 78-5286	35.8	2755.2
EHJU 1P	36.8	1899.7
COLQUITT	37.0	2943.8
PIONEER 9711	37.6	3044.8
LA 79-1004	40.2	2337.5
COKER 6727	43.6	2175.8
DPX 878	47.1	2189.3
PIONEER 9751	52.8	2007.4
LA 87-7030	59.0	1744.7
TERRAVIG 708	60.2	1778.4
TERRAVIG 727	60.9	1919.9
$LSD_{.05}$	31.6	862.3

1/ Average percent of plants with perithecia from random plot samples of 1 m lengths of row.

Table 5.9. Red crown rot incidence and yield (Kg/Ha) of maturity group IV cultivars, Iberia Research Station, Jeanerette, LA 1990.

Cultivar	Red crown rot[1] Incidence (%)	Yield Kg/Ha
S48-84	0.0	3222
AT 495	2.8	1275
RINGAROUND 452	3.7	3534
STEVENS	4.8	2654
CRAWFORD	5.0	2384
ASGRO 4906	6.2	3423
ASGRO 4595	6.6	2592
AVERY	6.7	3361
RIVERSIDE 499	7.3	4248
PIONEER 9391	7.7	1483
DEKALB CX458	8.5	2384
HARTZ 4464	8.8	2682
PIONEER 9411	14.0	1497
DEKALB CX415	14.1	1871
PIONEER 9442	14.6	1774
WILLIAMS	15.0	2162
LSD .05	8.6	

1/ Average percent of plants with perithecia from random plot samples of 1 m lengths of row.

Table 5.10. Red crown rot incidence and yield (Kg/Ha) of maturity groups V - VII cultivars, Robert Farm, Burnside, LA 1990.

Cultivar	Maturity Group	Red crown rot[1] Incidence (%)	Yield Kg/Ha
THOMAS	VII	12.9	3666
BRAXTON	VII	13.5	3902
PIONEER 9791	VII	17.3	3853
ASGRO 7986	VII	18.1	4012
HARTZ 7190	VII	22.2	3971
ASGRO 6297	VI	22.7	3611
AGRI TECH 550	V	23.4	2904
AGRI TECH 700	VII	23.7	3125
DAVIS	VI	24.3	3472
BRYAN	VI	24.5	3250
COKER 686	VI	25.0	2966
B2J	VI	25.3	3077
S64-23	VI	25.5	2814
DELTAPINE 415	V	26.0	3292
ASGRO 5979	V	26.7	3410
FFR 565	V	27.5	2557
STONEWALL	VII	29.1	3735
WALTERS	V	29.5	3063
PIONEER 9591	V	30.5	3444
CAJUN	VI	30.7	3437
HARTZ 7126	VII	31.2	2952
HUTCHESON	V	31.3	3534

Table 5.10. continued

Cultivar	Maturity Group	Red crown rot[1] Incidence (%)	Yield Kg/Ha
ASGRO 7258	VII	31.4	3763
LAMAR	VI	31.8	2730
PIONEER 9581	V	32.1	2862
BRIM	VI	32.4	3583
PIONEER 9711	VII	32.7	2918
PIONEER 9751	VII	32.8	3375
PIONEER 9592	V	33.6	3209
CENTENNIAL	VI	33.8	2966
HARTZ 7585	VII	33.9	3541
COKER 6847	VII	35.0	3354
VICTORY	VI	35.5	2675
BUCKSHOT 703	VII	35.8	2626
RALLY	VII	37.9	3077
CORDELL	V	41.5	2176
FFR 561	V	41.7	3278
HARTZ 5370	V	42.7	2883
ASGRO 6785	VI	42.8	2717
AGRI TECH 575	V	43.7	2897
FORREST	V	47.0	2495
HSC 721	VII	48.1	3534
FFR STONE	V	49.1	2453
$LSD_{.05}$		22.0	589

1/ Average percent of plants with perithecia from random plot samples of 1 m lengths of row.

5.9). In the case of all of the tables, caution should be used when making any untested comparison between tables, since each table represents a separate analysis. During the 1989 growing season at the Iberia Research Station no RCR was observed in the blocks planted to maturity groups IV and V. During 1990, only the maturity group IV block at this location showed any substantial disease development. RCR data was not collected at the Burnside location in 1989 or at the St. Gabriel location in 1990 because of poor stand establishment.

Data from 1985 (Tables 5.1, 5.2) showed relatively low percentages of RCR using the newly developed rating system. This was probably the result of both low incidence and lack of proficiency of the researcher in identifying C. crotalariae infected plants. Despite the low percentages, there were clear differences in RCR incidence among cultivars. The data from Tables 5.1 and 5.2 in which the most confidence is placed are identifications of the extremely susceptible cultivars ('Bragg', 'Forrest', 'Shiloh', and 'Hartz 6383') the RCR reactions of which did not vary substantially over the years. The ranking of some of the more resistant cultivars ('Wright', 'Ransom', and 'Davis') are also accurate as indicated by data in other tables. However, 'Centennial' which ranks as somewhat resistant in Table 5.1 and 'Braxton' which ranks as somewhat susceptible in Table 5.2 are probably misclassified.

Data from 1986 (Table 5.3) reflect the more frequent ranking of the cultivars with 'Braxton' ranking as very resistant and 'Centennial', 'Forrest', and 'Hartz 6383' ranking as susceptible. Because of the aggregated distribution of the pathogen in the field there is a large amount of variability in all of the data. Consequently, some cultivars are ranked in an intermediate region between resistance and

susceptibility. When compared (Table 5.4) the maturity group VII cultivars were, as a group, significantly more resistant and higher yielding than either the group VI or V.

The pooled data from the three locations in 1988 (Table 5.5) showed several interesting results. First, the most resistant cultivar was of the early maturity group V. Second, the cultivar 'Braxton' which was resistant at the Burnside location was almost equal in reaction to 'Centennial' which was susceptible at the Burnside location. Note that, although Burnside is one of the locations contributing data to this overall mean, the contribution is only 1/3 (2 of 6 replications). Third, although the relative rankings of 'Braxton' and 'Centennial' have changed, 'Forrest' is still ranked as one of the more susceptible cultivars. Because of the large variability cultivars were significantly different from each other. However, the pattern of changing ranks does seem to be a salient point and will be discussed later. 'Asgro 7986' was the highest yielding material and had a good level of RCR resistance.

Although a maturity group V cultivar ranked as the most resistant in 1988, the overall effect of maturity was the same when data from 1985, 1986, and 1988 were analyzed together (Table 5.6). The maturity group VII cultivars were significantly more resistant and higher yielding than the other groups.

Because of the separate analyses of the maturity group blocks at the Iberia Research Station in 1989 direct comparisons of 'Braxton' and 'Centennial' were not possible (Tables 5.7, 5.8). 'Braxton' was moderately susceptible while 'Centennial' was susceptible. Although the

disease pressure in the maturity group VII and VIII block (over 60% RCR incidence in the more susceptible cultivar) was greater than the maturity group VI block, the most resistant cultivar was a maturity group VII - 'Hsc 721'. Within the maturity group VI block the more resistant cultivars were two 'Riverside' cultivars and 'Hartz 6200'.

In 1990, maturity group IV cultivars were evaluated for RCR reaction for the first time in these studies. The results of this evaluation are presented in Table 5.9. Overall there was low RCR incidence. It should be noted, however, that the other maturity group blocks at the Iberia Research Station in 1990 displayed insignificant levels of RCR.

Results of the 1990 cultivar trials at Burnside are presented in Table 5.10. 'Braxton' was once again one of the two most resistant cultivars while 'Centennial' was one of the more susceptible. The most striking result was the ranking of 'Hsc 721' as the second most susceptible when the previous year at the Iberia Research Station it was ranked the most resistant. 'Asgro 7986' was once again the highest yielding cultivar and possesses an apparently good level of RCR resistance. The other cultivars in the trial ranked approximately as before.

Compiled results for the years of the study for cultivars with two or more years data are presented in Tables 5.11, 5.12, and 5.13 for maturity groups V, VI, and VII, respectively. Because of the shifting positions in the ranking of some cultivars in different years, there is no ranked order in the tables. Because of unequal representation, there are few valid comparisons between the overall means of the cultivars, and inferences about overall merit should be applied cautiously.

Overall maturity group reaction to RCR for the course of the study

Table 5.11. Red crown rot incidence[1] and yield (Kg/Ha) of maturity group V cultivars for 1985, 1986, 1988, and 1990.

	1990		1988		1986		1985		Mean	
Cultivar	RCR	Yield	RCR	Yield	RCR	Yield	RCR	YIELD	RCR	YIELD
FFR 561	41.7	3186	15.3	2829	27.2	2837	----	----	28.0	2951
HARTZ 5370	42.7	2802	6.5	2944	----	----	----	----	24.6	2873
FORREST	47.0	2425	19.9	2735	37.1	2608	10.2	2243	28.6	2512
DELTAPINE 105	----	----	0.0	2923	24.1	3329	8.4	2782	10.8	3011
WILSTAR 550	----	----	11.0	2553	23.6	2630	6.9	2290	13.8	2491
COKER 485	----	----	12.1	2903	23.4	2773	----	----	17.8	2838
TERRAVIG 505	----	----	12.6	2883	15.4	2767	2.6	2486	10.2	2712
BEDFORD	----	----	17.4	1664	20.0	2551	5.5	2405	14.3	2207
HARTZ 5171	----	----	17.9	3031	41.0	2723	----	----	29.5	2877
ASGRO 5980	----	----	23.0	3112	32.9	2975	----	----	28.0	3044
DELTAPINE 345	----	----	----	----	21.0	2901	4.8	2465	12.9	2683
ASGRO 5474	----	----	----	----	24.9	2717	4.5	2600	14.7	2659
$LSD_{.05}$	22.0	8.5	16.8	1192	16.5	1045	10.5	525		

1/ Average percent of plants with perithecia from random plot samples of 1 m lengths of row.

Table 5.12. Red crown rot incidence[1] and yield (Kg/Ha) of maturity group VI cultivars for 1985, 1986, 1988, 1989, and 1990.

	1990		1989		1988		1986		1985		Mean	
Cultivar	RCR	Yield	RCR	Yield	RCR	Yield	RCR	YIELD	RCR	YIELD	RCR	YIELD
ASGRO 6297	22.7	3610	36.3	3001	----	----	----	----	----	----	29.5	3306
DAVIS	24.3	3472	20.5	2238	8.5	3839	26.3	3346	2.6	2486	16.4	3076
BUCKSHOT 603	----	----	20.6	2710	15.9	3153	----	----	----	----	18.3	2931
CAJUN	30.7	3437	29.7	3125	17.4	3832	----	----	----	----	25.9	3465
RINGAROUND 680	----	----	26.1	2710	19.5	3479	23.6	3377	6.0	3051	18.8	3154
SAMPSON	----	----	25.1	2495	11.4	3853	----	----	----	----	18.3	3174
TERRAVIG 616	----	----	24.1	2557	4.4	3604	----	----	----	----	14.3	3084
LAMAR	31.8	2730	40.9	2114	----	----	----	----	----	----	36.4	2425
CENTENNIAL	33.8	2966	49.1	2183	10.1	3333	25.6	3004	2.7	2897	24.3	2877
DELTAPINE 566	----	----	23.7	1982	6.1	3043	----	----	4.7	2640	9.9	2555
SHARKEY	----	----	23.0	3195	12.8	4068	----	----	----	----	17.9	3631
VICTORY	35.5	2668	----	----	5.8	1816	----	----	----	----	20.7	2245
ASGRO 6785	42.8	2717	21.0	3333	15.4	3812	----	----	----	----	26.4	3285
COKER 686	25.0	2966	----	----	25.0	3175	----	----	----	----	25.0	3070
B2J	25.3	3077	13.8	2848	----	----	----	----	----	----	19.6	2966

Table 5.12. continued

RINGAROUND 606	----	----	12.6	2911	19.4	3299	20.5	3448	----	----	17.5	3219
TRACY M	----	----	----	----	15.6	3314	17.2	3268	2.7	2452	11.8	3011
DELTAPINE 506	----	----	38.0	2263	17.1	3550	26.3	3333	2.8	3031	21.1	3044
TERRAVIG 606	----	----	----	----	----	----	22.3	3792	3.2	2647	12.8	3220
COKER 156	----	----	----	----	----	----	25.2	3188	6.4	2944	15.8	3066
HARTZ 6383	----	----	----	----	----	----	39.5	2701	15.4	2843	27.5	2772
$LSD_{.05}$	22.0	589	24.8	631	16.8	1227	16.5	1045	10.5	525		

1/ Average percent of plants with perithecia from random plot samples of 1 m lengths of row.

Table 5.13. Red crown rot incidence and yield (Kg/Ha) of maturity group VII cultivars for 1985, 1986, 1988, 1989, and 1990.

Cultivar	1990		1989		1988		1986		1985		MEAN	
	RCR	Yield	RCR	Yield	RCR	Yield	RCR	Yield	RCR	Yield	RCR	Yield
BRAXTON	13.5	3902	32.5	2439	8.5	3839	8.5	4277	6.7	3213	13.9	3534
PIONEER 9791	17.3	3853	26.0	2723	----	----	----	----	----	----	21.7	3292
ASGRO 7986	18.1	4012	30.3	2987	6.9	5378	----	----	----	----	24.2	4123
HARTZ 7190	22.2	3971	19.2	2904	----	----	----	----	----	----	20.7	3437
AGRI TECH 700	23.7	3125	26.9	2696	----	----	----	----	----	----	25.3	2911
STONEWALL	29.1	3735	31.8	2959	----	----	----	----	----	----	30.5	3347
HARTZ 7126	31.2	2952	33.6	2779	21.8	4151	28.8	3226	5.3	2977	24.1	3221
ASGRO 7258	31.4	3763	17.4	2710	----	----	----	----	----	----	24.4	3229
DELTAPINE 417	----	----	30.3	2987	19.0	3791	----	----	----	----	24.6	3389
PIONEER 9711	32.7	2918	37.6	3132	----	----	----	----	----	----	35.2	3028
PIONEER 9751	32.8	3375	52.8	2065	----	----	----	----	----	----	42.8	2723
HARTZ 7585	33.9	3541	23.2	3257	----	----	----	----	----	----	28.6	3403
COKER 6847	35.0	3354	----	----	5.8	4158	----	----	----	----	20.4	3756
BUCKSHOT 703	35.8	2626	35.3	2717	13.6	4130	----	----	----	----	28.2	3160
RALLY	37.9	3077	20.7	3222	----	----	----	----	----	----	29.3	3153

Table 5.13. continued

TERRAVIG 708	----	----	60.2	1830	12.0	3188	17.3	3395	5.2	2640	23.7	2763
COKER 6727	----	----	43.6	2238	7.1	3188	----	----	----	----	25.4	2717
HSC 721	48.1	3534	1.8	3728	----	----	----	----	----	----	24.6	3631
NKS 72-60	----	----	----	----	6.0	3301	12.9	2811	----	----	9.5	3056
WILSTAR 790	----	----	----	----	----	----	15.4	3390	5.7	3321	10.6	3356
RANSOM	----	----	----	----	----	----	18.6	3190	0.5	2304	9.6	2747
BRAGG	----	----	----	----	----	----	22.5	2999	15.2	3132	18.9	3066
COKER 237	----	----	----	----	----	----	24.3	3915	1.6	3065	13.0	3490
WRIGHT	----	----	----	----	----	----	27.7	3838	0.4	2964	14.1	3401
$LSD_{.05}$	22.0	589	31.6	887	16.8	1227	16.5	1045	7.8	525		

1/ Average percent of plants with perithecia from random plot samples of 1 m lengths of row.

is presented in Table 5.14. Based on all data, the maturity group VII cultivars were not significantly more resistant than either the group V or VI cultivars. The group VI cultivars were significantly (P=0.05) more resistant than the group V cultivars. When data from the two years with the most extensive data (1989 and 1990) were tested for correlations of yield and RCR reaction (Table 5.15) the correlation for maturity group VII was stronger than for group VI in 1989 and essentially the same in 1990.

Data from all of the above trials was assembled comprising 5,350 observations. When correlation analysis was run, there was a highly significant r value of 0.50 between numbers of plants bearing perithecia and planting density. When the data were analyzed by regression an r^2 of .25 was produced around the equation: numbers of plants with perithecia = -.489 + .251 x planting density. All terms in the equation are highly significant.

DISCUSSION

Because of the difficulties in assessing RCR based on leaf symptomoloy, the rating system based on presence/absence of perithecia at the base of the stem was the most accurate. The described method of randomly sampling plots was very objective, albeit time consuming, and gave unbiased results. As mentioned, the low RCR percentages in the 1985 trial probably reflected both low incidence and lack of rating expertise. Although bright red perithecia developed at the base of infected plants, there were dramatic plant-to-plant differences in numbers produced, distribution around the base of the stem, and brightness of pigmentation. Plants with few dull pigmented, dry perithecia that

Table 5.14. Least squares means of red crown rot incidence for soybean maturity groups V, VI, and VII average from 1986 to 1990.

Maturity Group	Red crown rot[1] Incidence (%)	Standard Error
V	27.2	1.66
VI	22.6	1.14
VII	24.1	1.28

1/ Average percent of plants with perithecia from random plot samples of 1 m lengths of row.

Table 5.15. Correlation coefficients (r) for yield vs. % red crown rot incidence for maturity groups V, VI, and VII soybeans in 1989 and 1990.

MATURITY GROUP	YEAR	
	1989	1990
V	----	$-.52^{.05}$
VI	$-.43^{.01}$	$-.50^{.10}$
VII	$-.78^{.01}$	$-.54^{.05}$

Superscript numbers: .01,.05,.10 are probabilities of greater absolute r values.

were largely present on the side of the stem opposite from that being observed were difficult to identify as infected. As the researcher became more experienced over the years of the study the data reflect less experimental variability.

The shifts in relative ranking of 'Braxton' and 'Centennial' among locations are of extreme interest. These shifts imply the possibility of soybean "race" differences within C. crotalariae. However, the race concept cannot be strictly applied in the case of quantitative resistance since there are no avirulent isolates or immune cultivars. The existence of races of C. crotalariae on peanut is a matter of some controversy (3,6,15). Black, et al. conclude that the quantitative resistance in peanut (expressed as reduced CBR incidence) is stable for heterogeneous populations of C. crotalariae (3) thus indicating the lack of "race" development. In soybean, however, this resistance may well not be stable with different populations. Adding to this line of evidence is information from the growers in Burnside and Maringouin. At the test site near Burnside, the producer will not grow 'Centennial' because he has suffered large losses to RCR on this cultivar. In cultivar trials on this property (Tables 5.1, 5.10) 'Centennial' was susceptible. Conversely, the producer at the Maringouin test site grows 'Centennial' almost exclusively because it yields well and does not appear overly susceptible to C. crotalariae. This cultivar appeared to be no more susceptible than 'Braxton' at this location (Table 5.5).

Additional evidence to support the race theory comes from the varying RCR reaction of 'Hsc 721' in trials conducted at the Iberia Research Station and at the Burnside location (Tables 5.8, 5.10). Because of the extremely low rating of this cultivar at the Iberia Re-

search Station, the plots were rechecked after rating to make sure that the plots were not an unusual series of escapes. Each of plot of 'Hsc 721' were virtually free of RCR and each was surrounded by plots in which RCR incidence was high. The cultivar appeared to be very resistant.

Although shifts in ranking of these three cultivars was the most extreme, there were others that also changed relative rank. The degree of change was not as great as that seen with the above cultivars and shift may be due to variability in experimental conditions rather than to any differences in the pathogen populations. However, further studies are necessary to elucidate these relationships.

When noting the distribution of the maturity groups in Table 5.3, the more resistant material appeared to be in the later maturity groups. This seemed to imply that resistance was linked to late maturity, a potentially undesirable situation, since resistant selections would be only of the late maturity group and growers would not be able to choose early maturing resistant cultivars. Fortunately, after testing all of the data, there does not appear to be any linkage between maturity group and resistance to RCR. Thus, resistant material can be selected without regard to maturity and resistant cultivars can be produced that will fit the diverse cropping system of the average grower.

Although a coefficient of determination of .25 for the regression of numbers of plants bearing perithecia on within-row planting density is not high when viewed absolutely, it is high when realized that 25% of the variation is accounted for solely by planting density. If cultivar differences and environmental differences were also accounted for,

the r^2 value would be expected to increase greatly. The cause of this relatively high correlation is probably spread of the disease by root-to-root contact between adjacent plants. As mentioned in the introduction, resistance to C. crotalariae in peanuts is thought to be due to quantitative differences in root periderm thickness (7,11). Periderm thickness is probably also a mechanism of resistance in soybean which is additionally governed by the probability of a suitable infection court and a germinating microsclerotia coming into contact. This probability, in turn, is governed by microsclerotia density and by extent of the host root system. Restricted lateral growth of the root system would minimize contact with microsclerotia that are concentrated in crop debris in the upper soil layers and reduce the amount of root-to-root spread of the disease. Restricted lateral root growth seems to be a viable and measureable second mechanism of resistance which could be selected. Additional research needs to be conducted to determine if selection and/or identification of cultivars or breeding material for restricted lateral root growth could be used to elevate RCR resistance.

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VITA

Dana Kent Berner was born in Pekin, Illinois on November 15, 1952. He graduated from Pekin Community High School in June, 1970, and subsequently attended Illinois State University where he received his B.S. degree in biology in 1975. From summer of 1975 to winter of 1979 he served as a Peace Corps volunteer in Sierra Leone West Africa working as a rice production specialist. During this time he traveled extensively throughout Africa conducting rice production training programs for indigenous personnel. After returning to the United States, he obtained a M.S. degree in Agronomy at Louisiana State University with his area of specialization in rice genetics. He is currently finishing work on his Ph.D. degree in the Department of Plant Pathology and Crop Physiology at Louisiana State University where he has also been working as an instructor conducting research on soybean diseases. During his studies at Louisiana State University, he has achieved a cumulative grade point average of 4.0 for all of his graduate work. He is married to Cielito Berner and they have a family of five children.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Dana Kent Berner

Major Field: Plant Health

Title of Dissertation: Distribution and Management of and Soybean Resistance to Calonectria crotalariae, the Causal Pathogen of Red Crown Rot of Soybean

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

April 18, 1991